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(57) Abstract																																																			
<p>Compositions and methods for the detection and therapy of cancer are disclosed. The compounds provided include human endogenous retroviral sequences that are preferentially expressed in tumor tissue, as well as polypeptides encoded by such nucleotide sequences. Vaccines and pharmaceutical compositions comprising such compounds are also provided and may be used, for example, for the prevention and treatment of cancer. The polypeptides may also be used for the production of antibodies, which are useful for diagnosing and monitoring the progression of cancer in a patient.</p>																																																			
<p style="text-align: center;">NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE BREAST-TUMOR SPECIFIC cDNA B18Ag1</p> <table border="0"> <tbody> <tr> <td>TTA GAG ACC CAA TTG GGA CCT AAT TGG GAC CCA AAT TTC TCA AGT GGA</td> <td>48</td> </tr> <tr> <td>Leu Glu Thr Gln Leu Gly Pro Asn Trp Asp Pro Asn Phe Ser Ser Gly</td> <td></td> </tr> <tr> <td>1 5 10 15</td> <td></td> </tr> <tr> <td>GGG AGA ACT TTT GAC GAT TTC CAC CGG TAT CTC CTC GTG GGT ATT CAG</td> <td>96</td> </tr> <tr> <td>Gly Arg Thr Phe Asp Asp Phe His Arg Tyr Leu Leu Val Gly Ile Gln</td> <td></td> </tr> <tr> <td>20 25 30</td> <td></td> </tr> <tr> <td>GGA GCT GCC CAG AAA CCT ATA AAC TTG TCT AAG GCG ATT GAA GTC GTC</td> <td>144</td> </tr> <tr> <td>Gly Ala Ala Gln Lys Pro Ile Asn Leu Ser Lys Ala Ile Glu Val Val</td> <td></td> </tr> <tr> <td>35 40 45</td> <td></td> </tr> <tr> <td>CAG GGG CAT GAT GAG TCA CCA GGA GTG TTT TTA GAG CAC CTC CAG GAG</td> <td>192</td> </tr> <tr> <td>Gln Gly His Asp Glu Ser Pro Gly Val Phe Leu Glu His Leu Gln Glu</td> <td></td> </tr> <tr> <td>50 55 60</td> <td></td> </tr> <tr> <td>GCT TAT CGG ATT TAC ACC CCT TTT GAC CTG GCA GCC CCC GAA AAT AGC</td> <td>240</td> </tr> <tr> <td>Ala Tyr Arg Ile Tyr Thr Pro Phe Asp Leu Ala Ala Pro Glu Asn Ser</td> <td></td> </tr> <tr> <td>65 70 75 80</td> <td></td> </tr> <tr> <td>CAT GCT CTT AAT TTG GCA TTT GTG GCT CAG GCA GCC CCA GAT AGT AAA</td> <td>288</td> </tr> <tr> <td>His Ala Leu Asn Leu Ala Phe Val Ala Gln Ala Ala Pro Asp Ser Lys</td> <td></td> </tr> <tr> <td>85 90 95</td> <td></td> </tr> <tr> <td>AGG AAA CTC CAA AAA CTA GAG GGA TTT TGC TGG AAT GAA TAC CAG TCA</td> <td>336</td> </tr> <tr> <td>Arg Lys Leu Gln Lys Leu Glu Gly Phe Cys Trp Asn Glu Tyr Gln Ser</td> <td></td> </tr> <tr> <td>100 105 110</td> <td></td> </tr> <tr> <td>GCT TTT AGA GAT AGC CTA AAA GGT TTT</td> <td>363</td> </tr> <tr> <td>Ala Phe Arg Asp Ser Leu Lys Gly Phe</td> <td></td> </tr> <tr> <td>115 120</td> <td></td> </tr> </tbody> </table>				TTA GAG ACC CAA TTG GGA CCT AAT TGG GAC CCA AAT TTC TCA AGT GGA	48	Leu Glu Thr Gln Leu Gly Pro Asn Trp Asp Pro Asn Phe Ser Ser Gly		1 5 10 15		GGG AGA ACT TTT GAC GAT TTC CAC CGG TAT CTC CTC GTG GGT ATT CAG	96	Gly Arg Thr Phe Asp Asp Phe His Arg Tyr Leu Leu Val Gly Ile Gln		20 25 30		GGA GCT GCC CAG AAA CCT ATA AAC TTG TCT AAG GCG ATT GAA GTC GTC	144	Gly Ala Ala Gln Lys Pro Ile Asn Leu Ser Lys Ala Ile Glu Val Val		35 40 45		CAG GGG CAT GAT GAG TCA CCA GGA GTG TTT TTA GAG CAC CTC CAG GAG	192	Gln Gly His Asp Glu Ser Pro Gly Val Phe Leu Glu His Leu Gln Glu		50 55 60		GCT TAT CGG ATT TAC ACC CCT TTT GAC CTG GCA GCC CCC GAA AAT AGC	240	Ala Tyr Arg Ile Tyr Thr Pro Phe Asp Leu Ala Ala Pro Glu Asn Ser		65 70 75 80		CAT GCT CTT AAT TTG GCA TTT GTG GCT CAG GCA GCC CCA GAT AGT AAA	288	His Ala Leu Asn Leu Ala Phe Val Ala Gln Ala Ala Pro Asp Ser Lys		85 90 95		AGG AAA CTC CAA AAA CTA GAG GGA TTT TGC TGG AAT GAA TAC CAG TCA	336	Arg Lys Leu Gln Lys Leu Glu Gly Phe Cys Trp Asn Glu Tyr Gln Ser		100 105 110		GCT TTT AGA GAT AGC CTA AAA GGT TTT	363	Ala Phe Arg Asp Ser Leu Lys Gly Phe		115 120	
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Description

COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF CANCER

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Technical Field

The present invention relates generally to the detection and therapy of cancer. The invention is more specifically related to nucleotide sequences that are preferentially expressed in a tumor tissue and to polypeptides encoded by such nucleotide sequences. The invention is more particularly related to nucleotide sequences comprising at least a portion of a human endogenous retroviral sequence that is preferentially expressed in a tumor tissue, and to polypeptides encoded by such nucleotide sequences. The nucleotide sequences and polypeptides may be used in vaccines and pharmaceutical compositions for the prevention and treatment of cancer. The polypeptides may also be used for the production of compounds, such as antibodies, useful for diagnosing and monitoring the progression of cancer in a patient.

Background of the Invention

In recent years, considerable research has been directed to the identification of tumor markers, which may be useful for the diagnosis of particular cancers, for predicting the outcome of the disease or for developing a therapy in a patient-specific manner. Such research has generally focused on oncogenes, which are normal cellular genes whose expression has been altered (e.g., by gene amplification, increased transcription, alteration of mRNA splicing or mutation within the coding region) such that otherwise normal cells assume neoplastic growth behavior. To date, however, the established markers have had a limited utility, and their use often leads to a result that is difficult to interpret.

Management of cancer currently relies on a combination of early diagnosis and aggressive treatment, which may include one or more of a variety of treatments such as surgery, radiotherapy, chemotherapy and hormone therapy. However, current diagnostic methods often fail to detect a cancer until the disease has progressed to a state that is difficult to treat, and existing treatments often have serious side effects. The high mortality observed among cancer patients indicates that improvements are needed in the diagnosis and treatment of the disease.

Accordingly, there is a need in the art for improved tumor markers, and methods for therapy and diagnosis of cancer. The present invention fulfills these needs and further provides other related advantages.

Summary of the Invention

Briefly stated, this invention provides compositions and methods for the diagnosis and therapy of cancer. In one aspect, isolated DNA molecules are provided, comprising: (a) a human endogenous retroviral sequence, wherein the retroviral sequence is preferentially expressed in a tumor tissue; (b) a variant of the human endogenous retroviral sequence that contains one or more nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% (preferably no more than 5%) of the nucleotide positions, such that the antigenic and/or immunogenic properties of the polypeptide encoded by the human endogenous retroviral sequence are retained; or (c) a nucleotide sequence encoding an epitope of a polypeptide encoded by at least one of the above sequences. Isolated DNA and RNA molecules comprising a nucleotide sequence complementary to a DNA molecule as described above are also provided.

In another aspect, the present invention provides an isolated DNA molecule encoding an epitope of a polypeptide, the polypeptide being encoded by: (a) a nucleotide sequence transcribed from the sequence of SEQ ID NO:11; or (b) a variant of the nucleotide sequence that contains one or more nucleotide substitutions, deletions, insertions and/or modifications at not more than 20% of the nucleotide positions, such that the antigenic and/or immunogenic properties of the polypeptide encoded by the nucleotide sequence are retained. Isolated DNA and RNA molecules comprising a nucleotide sequence complementary to a DNA molecule as described above are also provided.

In related aspects, the present invention provides recombinant expression vectors comprising a DNA molecule as described above and host cells transformed or transfected with such expression vectors.

In further aspects, polypeptides, comprising an amino acid sequence encoded by a DNA molecule as described above, and monoclonal antibodies that bind to such polypeptides are provided.

In another aspect, methods are provided for determining the presence of a cancer in a patient. In one embodiment, the method comprises detecting, within a biological sample obtained from a patient, a polypeptide as described above. In another embodiment, the method comprises detecting, within a biological sample, an RNA molecule encoding a polypeptide as described above. In yet another embodiment, the method comprises (a) intradermally injecting a patient with a polypeptide as described above; and (b) detecting an immune response on the patient's skin and therefrom detecting the presence of a cancer in the patient.

In a related aspect, diagnostic kits useful in the determination of breast cancer are provided. The diagnostic kits generally comprise one or more monoclonal antibodies as described above, and a detection reagent. Within another related aspect, the diagnostic kit comprises a first polymerase chain reaction primer and a second polymerase chain reaction primer, the first and second primers each comprising at least about 10 contiguous nucleotides of an RNA molecule encoding a polypeptide as described above. Within yet another related aspect, the diagnostic kit comprises at least one oligonucleotide probe, the probe comprising at least about 15 contiguous nucleotides of a DNA molecule as described above. In another aspect, the present invention provides methods for monitoring the progression of a cancer in a patient. In one embodiment, the method comprises: (a) detecting an amount, in a biological sample, of a polypeptide as described above; (b) subsequently repeating step (a); and (c) comparing the amounts of polypeptide detected in steps (a) and (b), and therefrom monitoring the progression of cancer in the patient. In another embodiment, the method comprises (a) detecting an amount, within a biological sample, of an RNA molecule encoding a polypeptide as described above; (b) subsequently repeating step (a); and (c) comparing the amounts of RNA molecules detected in steps (a) and (b), and therefrom monitoring the progression of cancer in the patient.

In other aspects, pharmaceutical compositions, which comprise a polypeptide as described above and a physiologically acceptable carrier, and vaccines, which comprise a polypeptide as described above and an immune response enhancer are provided.

In related aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as described above.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

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Brief Description of the Drawings

Figure 1 shows the differential display PCR products, separated by gel electrophoresis, obtained from cDNA prepared from normal breast tissue (lanes 1 and 2) and from cDNA prepared from breast tumor tissue from the same patient (lanes 3 and 4). The arrow indicates the band corresponding to B18Ag1.

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Figure 2 is a northern blot comparing the level of B18Ag1 mRNA in breast tumor tissue (lane 1) with the level in normal breast tissue.

Figure 3 shows the level of B18Ag1 mRNA in breast tumor tissue compared to that in various normal and non-breast tumor tissues as determined by RNase protection assays.

Figure 4 is a genomic clone map showing the location of additional retroviral sequences (provided in SEQ ID NO:3 - SEQ ID NO:10) relative to B18Ag1.

Figures 5A and 5B show the sequencing strategy, genomic organization, and predicted open reading frame for the retroviral element containing B18Ag1.

Figure 6 shows the nucleotide sequence of the representative human endogenous retroviral element B18Ag1.

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Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for the diagnosis, monitoring and therapy of cancer. The compositions described herein include polypeptides, nucleic acid sequences and antibodies. Polypeptides of the present invention generally comprise at least a portion of a protein that is encoded by a human endogenous retroviral sequence, wherein the human endogenous retroviral sequence is expressed at substantially greater levels in a human tumor tissue than in normal tissue (*i.e.*, the level of RNA encoding the polypeptide is at least two fold higher, and preferably at least five fold higher, in a tumor tissue than in normal tissue). Such sequences are said to be "preferentially expressed" in a tumor tissue. Any cancer characterized by increased expression of a human endogenous retroviral sequence within a tumor may be detected and/or treated according to the present invention. Representative cancers include breast cancer, prostate cancer, leukemia, lymphoma and Kaposi's sarcoma. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (and epitopes thereof) encoded by a human endogenous retroviral sequence.

Nucleic acid sequences of the subject invention generally comprise a DNA or RNA sequence that encodes a polypeptide as described above, or that is complementary to such a sequence. Antibodies are generally immune system proteins, or fragments thereof, that are capable of binding to a portion of a polypeptide as described above. Antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies.

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Polypeptides within the scope of this invention include, but are not limited to, polypeptides (and epitopes thereof) encoded by the human endogenous retroviral sequences described herein. Such sequences include the sequence designated

B18Ag1 (SEQ ID NO:1) as well as other sequences such as those recited in SEQ ID NO:3-SEQ ID NO:10, found within the retroviral genome containing B18Ag1 (SEQ ID NO:11). B18Ag1 has homology to the P30 gene of the endogenous human retroviral element S71, as described in Werner et al., *Virology* 174:225-238 (1990). As used
5 herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins encoded by a human endogenous retroviral element. A polypeptide comprising an epitope of a human endogenous retroviral element may consist entirely of the epitope, or may contain additional sequences. The additional sequences may be derived from the native protein or may be heterologous, and such sequences may (but
10 need not) possess immunogenic or antigenic properties.

An "epitope," as used herein is a portion of a polypeptide that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Epitopes may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993)
15 and references cited therein. Such techniques include screening polypeptides derived from the native polypeptide for the ability to react with antigen-specific antisera and/or T-cell lines or clones. An epitope of a polypeptide is a portion that reacts with such antisera and/or T-cells at a level that is similar to the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such screens may
20 generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. B-cell and T-cell epitopes may also be predicted via computer analysis. Polypeptides comprising an epitope of a polypeptide that is preferentially expressed in a tumor tissue (with or without additional amino acid
25 sequence) are within the scope of the present invention.

The compositions and methods of the present invention also encompass variants of the above polypeptides and nucleic acid sequences encoding such polypeptides. A polypeptide "variant," as used herein, is a polypeptide that differs from the native polypeptide in substitutions and/or modifications such that the antigenic
30 and/or immunogenic properties of the polypeptide are retained. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antisera and/or T-cells as described above. Nucleic acid variants may contain one or more substitutions, deletions, insertions and/or modifications such that the antigenic and/or immunogenic
35 properties of the encoded polypeptide are retained. One preferred variant of a human endogenous retroviral sequence, or an epitope thereof, is a variant that contains

nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% of the nucleotide positions within the native polypeptide sequence.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic or antigenic properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Human endogenous retroviral sequences that are expressed at substantially greater levels in a human tumor tissue than in normal tissue may be prepared using any of several techniques. For example, the human endogenous retroviral sequence designated B18Ag1 (Figure 6 and SEQ ID NO:1) may be cloned on the basis of its breast tumor specific expression, using differential display PCR. This technique compares the amplified products from poly A+ or total RNA template prepared from normal and breast tumor tissue. cDNA may be prepared by reverse transcription of RNA using a (dT)₁₂AG primer. Following amplification using the primer CCTCAACCTC (SEQ ID NO:13), a band corresponding to an amplified product specific to the tumor RNA may be cut out from a silver stained gel and subcloned into a suitable vector (e.g., the T-vector, Novagen, Madison, WI).

Alternatively, the B18Ag1 gene (or a portion thereof) may be amplified from human genomic DNA, or from breast tumor cDNA, via polymerase chain reaction. For this approach, B18Ag1 sequence-specific primers may be designed based on the sequence provided in SEQ ID NO:1, and may be purchased or synthesized. One suitable primer pair for amplification from breast tumor cDNA is (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:14) and (5'CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:15). An amplified portion of B18Ag1 may then be used to isolate

the full length gene from a human genomic DNA library or from a breast tumor cDNA library, using well known techniques such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1989). Other sequences within the retroviral genome containing B18Ag1, such as those recited in SEQ ID NO:3 - SEQ ID NO:10, may be similarly prepared by screening human genomic libraries using B18Ag1-specific sequences as probes.

Other human endogenous retroviral sequences that are expressed at substantially greater levels in a human tumor tissue than in normal tissue may be prepared using methods known to those of ordinary skill in the art. For example, such sequences may be identified using low stringency hybridization, followed by PCR to identify conserved motifs. The level of expression in tumor tissue may generally be evaluated using the methods described herein, such as PCR and Northern blot analysis.

Recombinant polypeptides encoded by the DNA sequences described above may be readily prepared from the DNA sequences. For example, supernatants from suitable host/vector systems which secrete recombinant polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

In general, any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO.

Such techniques may also be used to prepare polypeptides comprising epitopes or variants of the native polypeptides. For example, variants of a native polypeptide may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides. Portions and other variants having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing

amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc., Foster City, CA, and may be operated according to the manufacturer's instructions.

5 In specific embodiments, polypeptides of the present invention encompass polypeptides encoded by a human endogenous retroviral sequence that is expressed at substantially greater levels in a human tumor tissue than in normal tissue (such as the sequence recited in SEQ ID NO:1), variants of such polypeptides that are encoded by DNA molecules containing one or more nucleotide substitutions, deletions,
10 insertions and/or modifications at no more than 20% of the nucleotide positions, and epitopes of the above polypeptides. Polypeptides within the scope of the present invention also include polypeptides (and epitopes thereof) encoded by DNA sequences that hybridize to the above sequences under stringent conditions, wherein the DNA sequences are at least 80% identical in overall sequence to the sequence recited in SEQ
15 ID NO:1, and wherein RNA corresponding to said nucleotide sequence is expressed at a greater level in human tumor tissue than in the corresponding normal tissue. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing overnight at 65°C in 6X SSC, 0.2% SDS; followed by washing twice at 65°C for 30 minutes each with 1X SSC, 0.1% SDS, and then washing twice at 65°C for 30-
20 60 minutes each with 0.1X SSC, 0.1% SDS. DNA molecules according to the present invention include molecules that encode any of the above polypeptides.

In another aspect of the present invention, antibodies are provided. Such antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory
25 Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for
30 polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity
35 chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J.*

Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Antibodies may be used, for example, in methods for detecting a cancer (such as breast cancer, prostate cancer, leukemia, lymphoma or Kaposi's sarcoma) in a patient. Such methods involve using one or more antibodies to detect the presence or absence of a polypeptide as described herein in a suitable biological sample. As used herein, suitable biological samples include tumor or normal tissue biopsy, mastectomy, blood, lymph node, serum and urine samples or other tissue, homogenate or extract thereof, obtained from a patient. It will be evident to those of ordinary skill in the art that, following detection of a polypeptide within a non-biopsy sample, additional tumor markers may be employed to identify the particular type of cancer.

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect polypeptide markers in a sample. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein preparation from the biological sample is submitted to gel electrophoresis, transferred to a suitable membrane and allowed to react with antibody. The presence of

antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of an antibody immobilized on a solid support to bind to the polypeptide and remove it from the remainder of the sample. The bound polypeptide may then be detected using a second antibody that binds to the binding partner/polypeptide complex and contains a reporter group. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result is indicative of the concentration of polypeptide in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose filter or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the antibody, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of antibody ranging from about 10 ng to about 1 μ g, and preferably about 100-200 ng, is sufficient to immobilize an adequate amount of polypeptide.

Covalent attachment of antibody to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the antibody. For example, the antibody may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde

group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.,* Pierce Immunotechnology Catalog and Handbook (1991) at A12-A13).

In certain embodiments for detection of polypeptide in a sample, the assay is a two-antibody sandwich assay. This assay may be performed by first
5 contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that the polypeptide within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a second antibody (containing a reporter group) capable of binding to a different site on the polypeptide is
10 added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such
15 as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.,* incubation time) is that period of time that is sufficient to detect the presence
20 of polypeptide within a sample obtained from an individual with breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of
25 time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support.
30 Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of antibody to reporter group may be achieved using standard methods known to those of ordinary skill in the art.

The second antibody is then incubated with the immobilized antibody-
35 polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound second antibody is then removed.

and bound second antibody is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value may be considered positive for a cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, p. 106-7 (Little Brown and Co., 1985). Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the antibody is immobilized on a membrane, such as nitrocellulose. In the flow-through test, the polypeptide within the sample binds to the immobilized antibody as the sample passes through the membrane. A second, labeled antibody then binds to the antibody-polypeptide complex as a solution containing the second antibody flows through the membrane. The detection of bound second antibody may then be performed as described above. In the strip test format, one end of the membrane to which antibody is bound is immersed in a solution containing the sample.

The sample migrates along the membrane through a region containing second antibody and to the area of immobilized antibody. Concentration of second antibody at the area of immobilized antibody indicates the presence of breast cancer. Typically, the concentration of second antibody at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of antibody immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 1 µg. Such tests can typically be performed with a very small amount of biological sample.

The presence or absence of a cancer in a patient may also be determined by evaluating the level of mRNA encoding a polypeptide of the present invention within the biological sample (e.g., a biopsy, mastectomy and/or blood sample from a patient) relative to a predetermined cut-off value. Such an evaluation may be achieved using any of a variety of methods known to those of ordinary skill in the art such as, for example, *in situ* hybridization and amplification by polymerase chain reaction. For example, polymerase chain reaction may be used to amplify sequences from cDNA prepared from RNA that is isolated from one of the above biological samples. Sequence-specific primers for use in such amplification may be designed based on a cDNA or genomic sequence, such as a sequence provided in SEQ ID NO:1 or SEQ ID NO:3 - SEQ ID NO:10, and may be purchased or synthesized. In the case of B18Ag1, as noted herein, one suitable primer pair is (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:14) and (5'CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:15). The PCR reaction products may then be separated and visualized using gel electrophoresis, according to methods well known to those of ordinary skill in the art. Amplification is typically performed on samples obtained from matched pairs of tissue (tumor and non-tumor tissue from the same individual) or from unmatched pairs of tissue (tumor and non-tumor tissue from different individuals). The amplification reaction is preferably performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the tumor sample as compared to the same dilution of the non-tumor sample is considered positive.

Conventional RT-PCR protocols using agarose and ethidium bromide staining, while important in defining gene specificity do not lend themselves to diagnostic kit development because of the time and effort required in making them

quantitative (*i.e.*, construction of saturation and/or titration curves), and their sample throughput. This problem is overcome by the development of procedures such as real time RT-PCR which allows for assays to be performed in single tubes, and in turn can be modified for use in 96 well plate formats. Instrumentation to perform such
5 methodologies are available from ABI/Perkin Elmer. Alternatively, other high throughput assays using labelled probes (*e.g.*, digoxigenin) in combination with labelled (*e.g.*, enzyme fluorescent, radioactive) antibodies to such probes can also be used in the development of 96 well plate assays.

In yet another method for determining the presence or absence of a
10 cancer in a patient, one or more of the polypeptides described above may be used in a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling, reddening or dermatitis) is measured following intradermal injection of one or more polypeptides as described above. Such injection may be achieved using any suitable device sufficient
15 to contact the polypeptide or polypeptides with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. Preferably, the reaction is measured at least 48 hours after injection, more preferably 48-72 hours.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to a test antigen (*i.e.*, an immunogenic
20 portion of a polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of a cancer. As noted above, additional tumor markers may be employed, using methods known to those of ordinary skill in the art, to identify the type of cancer
25 present.

The polypeptides of this invention are preferably formulated, for use in a skin test, as pharmaceutical compositions containing at least one polypeptide and a physiologically acceptable carrier, such as water, saline, alcohol, or a buffer. Such compositions typically contain one or more of the above polypeptides in an amount
30 ranging from about 1 µg to 100 µg, preferably from about 10 µg to 50 µg in a volume of 0.1 mL. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

In other aspects of the present invention, the progression and/or response to treatment of a cancer may be monitored by performing any of the above assays over
35 a period of time, and evaluating the change in the level of the response (*i.e.*, the amount of polypeptide or mRNA detected or, in the case of a skin test, the extent of the immune response detected). For example, the assays may be performed every 1-2 months for a

period of 1-2 years. In general, a cancer is progressing in those patients in whom the level of the response increases over time. In contrast, a cancer is not progressing when the signal detected either remains constant or decreases with time.

In further aspects of the present invention, the compounds described
5 herein may be used for the immunotherapy of a cancer. In these aspects, the compounds (which may be polypeptides, antibodies or nucleic acid molecules) are preferably incorporated into pharmaceutical compositions or vaccines. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more polypeptides and an immune response
10 enhancer, such as an adjuvant or a liposome (into which the compound is incorporated). Pharmaceutical compositions and vaccines may additionally contain a delivery system, such as biodegradable microspheres which are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109. Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, including one or
15 more separate polypeptides.

Alternatively, a vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. In such vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and
20 viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be
25 introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749 (1993) and reviewed by
30 Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier
35 will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier,

such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention.

5 Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, *Bordella pertussis* or *Mycobacterium tuberculosis*-derived proteins. Suitable
10 adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI), Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ), alum, biodegradable microspheres, monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

15 The above pharmaceutical compositions and vaccines may be used, for example, for the therapy of cancer in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with a cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer.
20 To prevent the development of a cancer, a pharmaceutical composition or vaccine comprising one or more polypeptides as described herein (or naked, plasmid or viral vector DNA encoding such a polypeptide) may be administered to a patient. For treating a patient with a cancer, the pharmaceutical composition or vaccine may comprise one or more polypeptides, antibodies or nucleic acid molecules
25 complementary to DNA encoding a polypeptide as described herein (*e.g.*, antisense RNA or antisense deoxyribonucleotide oligonucleotides).

For example, tumor cells that express a polypeptide as described herein may be preferentially killed by administering to a patient a conjugate in which a cytotoxic agent or "prodrug" is linked to antisense RNA, an antisense
30 deoxyribonucleotide oligonucleotide or an antibody that binds to such a polypeptide. As used herein, the term "prodrug" refers to a group that is not itself toxic to the cell, but that can be rendered toxic after the conjugate is directed to the target cell by the addition of a second activating compound, such as an enzyme that can convert the prodrug into an active drug. Any suitable cytotoxic agent (including radionuclides) or
35 prodrug known to those of ordinary skill in the art may be employed in such methods. Suitable prodrugs include boron, doxifluridine, or the prodrug precursor of palytoxin.

Routes and frequency of administration, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1
5 and 10 doses may be administered for a 52 week period. Preferably, 6 doses are administered, at intervals of one month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response. Such a response can
10 be monitored by measuring the level of anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. A suitable dose should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-
15 vaccinated patients.. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 100 μ g to about 5 mg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

20 The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1Preparation of B18Ag1 cDNA and Genomic Clones Using Differential Display RT-PCR

This Example illustrates the preparation of cDNA and genomic DNA molecules encoding B18Ag1 using a differential display screen.

Tissue samples were prepared from breast tumor and normal tissue of a patient with breast cancer that was confirmed by pathology after removal from the patient. Normal RNA and tumor RNA was extracted from the samples and mRNA was isolated and converted into cDNA using a (dT)₁₂AG anchored 3' primer. Differential display PCR was then executed using a randomly chosen primer (CTTCAACCTC) (SEQ ID NO:16). Amplification conditions were standard buffer containing 1.5 mM MgCl₂, 20 pmol of primer, 500 pmol dNTP, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer, Branchburg, NJ). Forty cycles of amplification were performed using 94°C denaturation for 30 seconds, 42°C annealing for 1 minute, and 72°C extension for 30 seconds. An RNA fingerprint containing 76 amplified products was obtained. Although the RNA fingerprint of breast tumor tissue was over 98% identical to that of the normal breast tissue, a band was repeatedly observed to be specific to the RNA fingerprint pattern of the tumor. This band was cut out of a silver stained gel and subcloned into the T-vector (Novagen, Madison, WI) and sequenced.

The sequence of the cDNA, referred to as B18Ag1, is provided in SEQ ID NO:1. A database search of GENBANK and EMBL revealed that the B18Ag1 fragment initially cloned is 77% identical to the endogenous human retroviral element S71, which is a truncated retroviral element homologous to the Simian Sarcoma Virus (SSV). S71 contains a complete *gag* gene, a portion of the *pol* gene and an LTR-like structure at the 3' terminus (*see* Werner et al., *Virology* 174:225-238 (1990)). B18Ag1 is also 64% identical to SSV in the region corresponding to the P30 (*gag*) locus. B18Ag1 contains three separate and incomplete reading frames covering a region which shares considerable homology to a wide variety of *gag* proteins of retroviruses which infect mammals. In addition, the homology to S71 is not just within the *gag* gene, but spans several kb of sequence including an LTR.

B18Ag1-specific PCR primers were synthesized using computer analysis guidelines. RT-PCR amplification (94°C, 30 seconds; 60°C → 42°C, 30 seconds; 72°C, 30 seconds, for 40 cycles) confirmed that B18Ag1 represents an actual mRNA sequence present at relatively high levels in the patient's breast tumor tissue.

The primers used in amplification were B18Ag1-1 (CTG CCT GAG CCA CAA ATG) (SEQ ID NO:17) and B18Ag1-4 (CCG GAG GAG GAA GCT AGA GGA ATA) (SEQ ID NO:18) at a 3.5 mM magnesium concentration and a pH of 8.5, and B18Ag1-2 (ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:14) and B18Ag1-3 (CCG
 5 GTA TCT CCT CGT GGG TATT) (SEQ ID NO:15) at 2 mM magnesium at pH 9.5. The same experiments showed exceedingly low to nonexistent levels of expression in this patient's normal breast tissue (*see* Figure 1). RT-PCR experiments were then used to show that B18Ag1 mRNA is present in nine other breast tumor samples (from Brazilian and American patients) but absent in, or at exceedingly low levels in, the
 10 normal breast tissue corresponding to each cancer patient. RT-PCR analysis has also shown that the B18Ag1 transcript is not present in various normal tissues (including lymph node, myocardium and liver) and present at relatively low levels in PBMC and lung tissue. The presence of B18Ag1 mRNA in breast tumor samples, and its absence from normal breast tissue, has been confirmed by Northern blot analysis, as shown in
 15 Figure 2.

The differential expression of B18Ag1 in breast tumor tissue was also confirmed by RNase protection assays. Figure 3 shows the level of B18Ag1 mRNA in various tissue types as determined in four different RNase protection assays. Lanes 1-12 represent various normal breast tissue samples, lanes 13-25 represent various breast
 20 tumor samples; lanes 26-27 represent normal prostate samples; lanes 28-29 represent prostate tumor samples; lanes 30-32 represent colon tumor samples; lane 33 represents normal aorta; lane 34 represents normal small intestine; lane 35 represents normal skin, lane 36 represents normal lymph node; lane 37 represents normal ovary; lane 38 represents normal liver; lane 39 represents normal skeletal muscle; lane 40 represents a
 25 first normal stomach sample, lane 41 represents a second normal stomach sample; lane 42 represents a normal lung; lane 43 represents normal kidney; and lane 44 represents normal pancreas. Interexperimental comparison was facilitated by including a positive control RNA of known B-actin message abundance in each assay and normalizing the results of the different assays with respect to this positive control.

30 RT-PCR and Southern blot analysis has shown the B18Ag1 locus to be present in human genomic DNA as a single copy endogenous retroviral element. A genomic clone of approximately 12-18 kb was isolated using the initial B18Ag1 sequence as a probe. Four additional subclones were also isolated by XbaI digestion. Additional retroviral sequences obtained from these clones (located as shown in Figure
 35 4) are shown as SEQ ID NO:3 - SEQ ID NO:10, where SEQ ID NO:3 shows the location of the sequence labeled 10 in Figure 4, SEQ ID NO:4 shows the location of the sequence labeled 11-29, SEQ ID NO:5 shows the location of the sequence labeled 3,

SEQ ID NO:6 shows the location of the sequence labeled 6, SEQ ID NO:7 shows the location of the sequence labeled 12, SEQ ID NO:8 shows the location of the sequence labeled 13, SEQ ID NO:9 shows the location of the sequence labeled 14 and SEQ ID NO:10 shows the location of the sequence labeled 11-22.

5 Subsequent studies demonstrated that the 12-18 kb genomic clone contains a retroviral element of about 7.75 kb, as shown in Figures 5A and 5B. The sequence of this retroviral element is shown in SEQ ID NO:11. The numbered line at the top of Figure 5A represents the sense strand sequence of the retroviral genomic clone. The box below this line shows the position of selected restriction sites. The
10 arrows depict the different overlapping clones used to sequence the retroviral element. The direction of the arrow shows whether the single-pass subclone sequence corresponded to the sense or anti-sense strand. Figure 5B is a schematic diagram of the retroviral element containing B18Ag1 depicting the organization of viral genes within the element. The open boxes correspond to predicted reading frames, starting with a
15 methionine, found throughout the element. Each of the six likely reading frames is shown, as indicated to the left of the boxes, with frames 1-3 corresponding to those found on the sense strand.

Using the cDNA of SEQ ID NO:1 as a probe, a longer cDNA was obtained (SEQ ID NO:12) which contains minor nucleotide differences (less than 1%)
20 compared to the genomic sequence shown in SEQ ID NO:11.

Example 2

Preparation of B18Ag1 DNA from Human Genomic DNA

25 This example illustrates the preparation of B18Ag1 DNA by amplification from human genomic DNA.

B18Ag1 DNA may be prepared from 250 ng human genomic DNA using 20 pmol of B18Ag1 specific primers, 500 pmol dNTPS and 1 unit of *Taq* DNA polymerase (Perkin Elmer, Branchburg, NJ) using the following amplification
30 parameters: 94°C denaturing for 30 seconds, 30 second 60°C to 42°C touchdown annealing in 2°C increments every two cycles and 72°C extension for 30 seconds. The last increment (a 42°C annealing temperature) should cycle 25 times. Primers (B18Ag1-1, B18Ag1-2, B18Ag1-3 and B18Ag1-4) were selected using computer analysis. Primers synthesized were. Primer pairs that may be used are 1+3, 1+4, 2+3,
35 and 2+4.

Following gel electrophoresis, the band corresponding to B18Ag1 DNA may be excised and cloned into a suitable vector.

Example 3

Preparation of B18Ag1 DNA from Breast Tumor cDNA

5 This example illustrates the preparation of B18Ag1 DNA by amplification from human breast tumor cDNA.

 First strand cDNA is synthesized from RNA prepared from human breast tumor tissue in a reaction mixture containing 500 ng poly A+ RNA, 200 pmol of the primer (T)12AG (*i.e.*, TTT TTT TTT TTT AG) (SEQ ID NO:19), 1X first strand
10 reverse transcriptase buffer, 6.7 mM DTT, 500 mmol dNTPs, and 1 unit AMV or MMLV reverse transcriptase (from any supplier, such as Gibco-BRL (Grand Island, NY)) in a final volume of 30 μ l. After first strand synthesis, the cDNA is diluted approximately 25 fold and 1 μ l is used for amplification as described in Example 2. While some primer pairs can result in a heterogeneous population of transcripts, the
15 primers B18Ag1-2 (5'ATG GCT ATT TTC GGG GGC TGA CA) (SEQ ID NO:14) and B18Ag1-3 (5'CCG GTA TCT CCT CGT GGG TAT T) (SEQ ID NO:15) yield a single 151 bp amplification product.

Example 4

Identification of B-cell and T-cell Epitopes of B18Ag1

20 This Example illustrates the identification of B18Ag1 epitopes.

 The B18Ag1 sequence can be screened using a variety of computer algorithms. To determine B-cell epitopes, the sequence can be screened for
25 hydrophobicity and hydrophilicity values using the method of Hopp, *Prog. Clin. Biol. Res.* 172B:367-77 (1985) or, alternatively, Cease et al., *164 J. Exp. Med.* 1779-84 (1986) or Spouge et al., *J. Immunol.* 138:204-12 (1987). Additional Class II MHC (antibody or B-cell) epitopes can be predicted using programs such as AMPHI (*e.g.*, Margalit et al., *J. Immunol.* 138:2213 (1987)) or the methods of Rothbard and Taylor
30 (*e.g.*, *EMBO J.* 7:93 (1988)).

 Once peptides (15-20 amino acids long) are identified using these techniques, individual peptides can be synthesized using automated peptide synthesis equipment (available from manufacturers such as Applied BioSystems, Inc., Foster City, CA) and techniques such as Merrifield synthesis. Following synthesis, the
35 peptides can be used to screen sera harvested from either normal or breast cancer patients to determine whether patients with breast cancer possess antibodies reactive with the peptides. Presence of such antibodies in breast cancer patient would confirm the

immunogenicity of the specific B-cell epitope in question. The peptides can also be tested for their ability to generate a serologic or humoral immune in animals (mice, rats, rabbits, chimps etc.) following immunization *in vivo*. Generation of a peptide-specific antiserum following such immunization further confirms the immunogenicity of the specific B-cell epitope in question.

To identify T-cell epitopes, the B18Ag1 sequence can be screened using different computer algorithms which are useful in identifying 8-10 amino acid motifs within the B18Ag1 sequence which are capable of binding to HLA Class I MHC molecules. (see, e.g., Rammensee et al., *Immunogenetics* 41:178-228 (1995)). Following synthesis such peptides can be tested for their ability to bind to class I MHC using standard binding assays (e.g., Sette et al., *J. Immunol.* 153:5586-92 (1994)) and more importantly can be tested for their ability to generate antigen reactive cytotoxic T-cells following *in vitro* stimulation of patient or normal peripheral mononuclear cells using, for example, the methods of Bakker et al., *Cancer Res.* 55:5330-34 (1995); Vissczen et al., *J. Immunol.* 154:3991-98 (1995); Kawakami et al., *J. Immunol.* 154:3961-68 (1995); and Kast et al., *J. Immunol.* 152:3904-12 (1994). Successful *in vitro* generation of T-cells capable of killing autologous (bearing the same class I MHC molecules) tumor cells following *in vitro* peptide stimulation further confirms the immunogenicity of the B18Ag1 antigen. Furthermore, such peptides may be used to generate murine peptide and B18Ag1 reactive cytotoxic T-cells following *in vivo* immunization in mice rendered transgenic for expression of a particular human MHC Class I haplotype (Vitiello et al., *J. Exp. Med.* 173:1007-15 (1991)).

A representative a list of predicted B18Ag1 B-cell and T-cell epitopes, broken down according to predicted HLA Class I MHC binding antigen, is shown below:

Predicted Th Motifs (B-cell epitopes)

SSGGRTFDDFHRVLLVGI (SEQ ID NO:20)

QGAAQKPINLSKXIEVVQGHDE (SEQ ID NO:21)

SPGVFLEHLQEAYRIYTPFDLSA (SEQ ID NO:22)

Predicted HLA A2.1 Motifs (T-cell epitopes)

YLLVGIQGA (SEQ ID NO:23)

GAAQKPINL (SEQ ID NO:24)

NLSKXIEVV (SEQ ID NO:25)

EVVQGHDES (SEQ ID NO:26)

HLQEAYRIY (SEQ ID NO:27)

NLAFVAQAA (SEQ ID NO:28)

FVAQAAPDS (SEQ ID NO:29)

5 From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Corixa Corporation

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR THE TREATMENT
AND DIAGNOSIS OF CANCER

(iii) NUMBER OF SEQUENCES: 29

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SEED and BERRY LLP
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 10-JAN-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Maki, David J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 210121.418PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (206) 622-4900

(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 415 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGANTGTCA AAAACCTTNT AGGCTATCTC TAAAAGCTGA CTGGTATTCA TTCCAGCAAA	60
ATCCCTCTAG TTTTGGAGT TTCCTTTTAC TATCTGGGGC TGCCTGAGCC ACAAATGCCA	120
AATTAAGAGC ATGGCTATTT TCGGGGGCTG ACAGGTCAAA AGGGGTGTAA ATCCGATAAG	180
CCTCCTGGAG GTGCTCTAAA AACACTCCTG GTGACTCATC ATGCCCCTGG ACGACTTCAA	240
TCGNCTTAGA CAAGTTTATA GGTTCCTGGG CAGTCCCTGA ATACCCACGA GGAGATACCG	300
GTGGAAATCG TCAAAAGTTC TCCCTCCACT TGAGAAATTT GGGTCCCAAT TAGGTCCCAA	360
TTGGGTCTCT AATCACTATT CCTCTAGCTT CCTCCTCCGG NCTATTGGTT GATGT	415

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Trp Asp Pro Asn Phe Ser Ser Gly Gly Arg Thr Phe Asp Asp Phe His
1 5 10 15

Arg Tyr Leu Leu Val Gly Ile Gln Gly Ala Ala Gln Lys Pro Ile Asn
 20 25 30

Leu Ser Lys Xaa Ile Glu Val Val Gln Gly His Asp Glu Ser Pro Gly
 35 40 45

Val Phe Leu Glu His Leu Gln Glu Ala Tyr Arg Ile Tyr Thr Pro Phe
 50 55 60

Asp Lys Ser Ala Pro Glu Asn Ser His Ala Leu Asn Leu Ala Phe Val
65 70 75 80

Ala Gln Ala Ala Pro Asp Ser Lys Arg Lys Leu Gln Lys Leu Glu Gly
 85 90 95

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1180 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

NCNNNNNTTA TGATTACGCC AAGCGNGCAA TTAACCCTCA CTAAAGGGAA CAAAAGCTGG	60
AGCTCCACCG CGGTGGCGGC CGCTAGAATC TTCATACCCC GAACTCTTGG GAAAACTTTA	120
ATCAGTCACC TACAGTCTAC CACCCATTTA GGAGGAGCAA AGCTACCTCA GCTCCTCCGG	180
AGCCGTTTTA AGATCCCCCA TCTTCAAAGC CTAACAGATC AAGCAGCTCT CCGGTGCACA	240
ACCTGCGCCC AGGTAAATGC CAAAAAAGGT CCTAAACCCA GCCCAGGCCA CCGTCTCCAA	300
GAAAACTCAC CAGGAGAAAA GTGGGAAATT GACTTTACAG AAGTAAACC ACACCGGGCT	360
GGGTACAAAT ACCTTCTAGT ACTGGTAGAC ACCTTCTCTG GATGGACTGA AGCATTTGCT	420
ACCAAAAACG AAACTGTCAA TATGGTAGTT AAGTTTTTAC TCAATGAAAT CATCCCTCGA	480
CGTGGGCTGC CTGTTGCCAT AGGGTCTGAT AATGGAACGG CCTTCGCCCTT GTCTATAGTT	540
TAATCAGTCA GTAAGGCGTT AAACATTCAA TGGAAGCTCC ATTGTGCCTA TCGACCCAGA	600
GCTCTGGGAA GTAGAACGCA TGAAGTGCAC CCTAAAAAAA CACTCTTACA AAATTAATCT	660
TAAAAACCGG TGTTAATTGT GTTAGTCTCC TTCCCTTAGC CCTACTTAGA GTTAAGGTGC	720
ACCCCTTACT GGGCTGGGTT CTTTACCTTT TGAAATCATN TTTNGGAAGG GGCTGCCTAT	780
CTTTNCTTAA CTAAAAAANG CCCATTTGGC AAAAATTTCN CAACTAATTT NTACGTNCCT	840
ACGTCTCCCC AACAGGTANA AAAATCTNCT GCCCTTTTCA AGGAACCATC CCATCCATTC	900

CTNAACAAAA GGCCTGCCNT TCTTCCCCCA GTTAACTNTT TTTTNTTAAA ATTCCCAAAA 960
AANGAACCNC CTGCTGGAAA AACNCCCCC TCCAANCCCC GGCCNAAGNG GAAGGTTCCC 1020
TTGAATCCCN CCCCCNCNA NGGCCCGGAA CCNTTAAANT NGTTCNNGG GGTNNGGCCT 1080
AAAAGNCCNA TTTGGTAAAC CTANAAATTT TTTCTTTTNT AAAAACCACN NTTTNTTTT 1140
TCTTAAACAA AACCTNTTT NTAGNANCNT ATTTCCCNCC 1180

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1163 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TNCTTTGATA CCCNAGCGTT CAATTAACCC TCACTAAAGG GAACAAAAGC TGGAGCTCCA 60
CCGCGGTGGC GGCCGCTCTA GAGCTGCGCC TGGATCCCGC CACAGTGAGG AGACCTGAAG 120
ACCAGAGAAA ACACAGCAAG TAGGCCCTTT AAATACTCA CCTGTGTTGT CTTCTAATTT 180
ATTCTGTTTT ATTTGTTTC CATCATTTTA AGGGGTAAA ATCATCTTGT TCAGACCTCA 240
GCATATAAAA TGACCCATCT GTAGACCTCA GGCTCCAACC ATACCCAAG AGTTGTCTGG 300
TTTTGTTTAA ATTACTGCCA GGTTTCAGCT GCAGATATCC CTGGAAGGAA TATTCCAGAT 360

TCCCTGAGTA GTTTCAGGT TAAATCCTA TAGGCTTCTT CTGTTTGTAG GAAGAGTTCC	420
TGTCAGAGAA AAACATGATT TTGGATTTTT AACTTTAATG CTTGTGAAAC GCTATAAAAA	480
AAATTTTCTA CCCCTAGCTT TAAAGTACTG TTAGTGAGAA ATTAAAATTC CTCAGGAGG	540
ATTAAACTGC CATTTTCAGTT ACCCTAATTC CAAATGTTTT GGTGGTTAGA ATCTTCTTTA	600
ATGTTCTTGA AGAAGTGTTT TATATTTTCC CATCNAGATA AATTCTCTCN CNCCTTNNTT	660
TTNTNTCTNN TTTTTTAAAA CGGANTCTTG CTCCGTTGTC CANGCTGGGA ATTTTNTTTT	720
GGCCAATCTC CGCTNCCTTG CAANAATNCT GCNTCCCAA ATTACCNCCT TTTTCCCACC	780
TCCACCCCN GGAATTACCT GGAATTANAG GCCCCNCCC CCCCCCGGC TAATTTGTTT	840
TTGTTTTTAG TAAAAACGG GTTTCCTGTT TTAGTTAGGA TGGCCCANNT CTGACCCNT	900
NATCNTCCCC CTCNGCCCTC NAATNTTNGG NNTANGGCTT ACCCCCCCN GNNGTTTTTC	960
CTCCATTNAA ATTTTCTNTG GANTCTTGAA TNNCGGGTTT TCCCTTTTAA ACCNATTTTT	1020
TTTTTNNNC CCCANTTTT NCCTCCCCCN TNTNTAANGG GGGTTTCCA ANCCGGGTCC	1080
NCCCCANGT CCCCAATTTT TCTCCCCCCT CCTCTTTTTT CTTNCCCCA AAANTCCTAT	1140
CTTTCCTNN AAATATCNAN TNT	1163

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1122 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

NNGGTCCNNC TCAAAGTCAN TATAGGGCGA ATTGGGTACC GGGCCCCCCC TCGAGGTCTGA	60
CGGTATCGAT AAGCTTGATA TCGAATTCCT GCAGCCCGGG GGATCCACTA GTTCTAGACC	120
AAGAAATGGA GGATTTTAGA GTGACTGATG ATTTCTCTAT CATCTGCAGT TAGTAAACAT	180
TCTCCACAGT TTATGCAAAA AGTAACAAAA CCACTGCAGA TGACAAACAC TAGGTAACAC	240
ACATACTATC TCCCAAATAC CTACCCACAA GCTCAACAAT TTAAACTGT TAGGATCACT	300
GGCTCTAATC ACCATGACAT GAGGTCACCA CCAACCATC AAGCGCTAAA CAGACAGAAT	360
GTTTCCACTC CTGATCCACT GTGTGGGAAG AAGCACCGAA CTTACCCACT GGGGGGCTG	420
CNTCANAANA AAAGCCCATG CCCCCGGGTN TNCCTTTNAA CCGGAACGAA TNAACCCACC	480
ATCCCCACAN CTCCTCTGTT CNTGGGCCCT GCATCTTG TG CCTCNTNTN CTTTNGGGGA	540
NACNTGGGGA AGGTACCCCA TTCNTTGAC CCCNCNANAA AACCCNGTG GCCCTTGCC	600
CTGATTCNCN TGGGCCTTTT CTCTTTTCCC TTTTGGGTTG TTAAATTCC CAATGTCCCC	660
NGAACCTCT CCNTNCTGCC CAAAACCTAC CTAAATTNCT CNCTANGNNT TTTCTTGGTG	720
TTNCTTTTCA AAGGTNACCT TNCCTGTTCA NNCCNACNA AAATTTNTTC CNTATNNTGG	780
NCCCNAAAA ANNNATCNC CCNAATTGCC CGAATTGGTT NGGTTTTTCC TNCTGGGGGA	840
AACCCTTTAA ATTTCCCCCT TGGCCGGCCC CCCTTTTTTC CCCCCTTNG AAGGCAGGNR	900

GGTTCTTCCC GAACTTCCAA TTNCAACAGC CNTGCCCATT GNTGAAACCC TTTTCCTAAA 960
ATTAAAAAAT ANCCGGTTNN GGNNGGCCTC TTTCCCCTCC NGGNGGGNNG NGAAANTCCT 1020
TACCCCNAAA AAGGTTGCTT AGCCCCNGT CCCCCTCCC CCNGGAAAAA TNAACCTTTT 1080
CNAAAAAAGG AATATAANTT TNCCACTCCT TNGTTCTCTT CC 1122

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1091 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

NCNNNCNTT TGTNAAAGAC CGNCAGTGAG CGCGCGTAAT ACGACTCACT ATAGGGCGAA 60
TTGGGTACCG GGCCCCCCT CGAGGTCGAC GGTATCGATA AGCTTGATAT CGAATTCCTG 120
CAGCCCGGGG GATCCACTAG TTCTAGAGCT CGCGGCCGCG AGCTCTAATA CGACTCACTA 180
TAGGGCGTCG ACTCGATCTC AGCTCACTGC AATCTCTGCC CCCGGGGTCA TGCATTCTC 240
CTGCCTCAGC CTTCCAAGTA GCTGGGATTA CAGGCGTGCA ACACCACACC CGGCTAATTT 300
TGTATTTTTA ATAGAGATGG GGTTTTCCCT TGTTGGCCAN NATGGTCTCN AACCCCTGAC 360
CTCNGTGAT CCCCCNCCC NNGANCTCN ACTGCTGGGG ATNNCCGNNN NNNNCCTCCC 420

NNCNCNNNNN NNCNCNNTCC NTNNTCCTTN CTCNNNNNNN NCNNTCNNTC CNNCTTCTCN	480
CCNNNTNTTN TCNNCNNCCN NCNNNCCNCN TCCCCNCNNN TTCNCNTNCN NTNTCCNNCN	540
NNNTCNNCNN NCNNNNCNTN NCCNNTACNT CNTNNNCNNN TCCNTCTNTN NCCTCNNCNN	600
TCNCTNCNCN TTNTCTCCTC NNTNNNNNNC TCCNNNNNTC TCNTCNCNC NTNCCTCNNT	660
NNCCNCNCCC CNCCTCNCNN CCTNNTTTTN NCNNCNNNTC CNTNCCNTTC NNNTCCNNTN	720
NCNNCNTCNC NNNCNTTNTT CCCNCCNNTT CCTTNCNCNT NNNNTNTCNN NCNCNTCNNT	780
CNTTTNCTCC TNNNTCCCN CTCNNTTCNC CCNNNTCCNC CCCCCNCCTN TCTCTNCCC	840
NNNTNNTNT NNNNCNTCCN CTNTCNCNTT CNTCNTNCN TTNCTNTCNN CNNCNTNCN	900
CTNCCNTNTN TCTNNNTCNC NTCNCNTNTC NCCNTCCNTT NCTNTCTCCT NTNTCCTTCC	960
CCTCNCCTNC TCNTTCNCCN CCCNNTNTNT NTNNCNCNN TNCTNNNCNN CCNTCNTTTC	1020
NTCTCTNCTN NNNNTNNCCT CNNCCCTNC CCTNNTNCNC TNCTNNTACC NTNCTNCTCC	1080
NTCTTCCTTC C	1091

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1165 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

NCNNNTTATG ATTACGCCNA CGNNCAATTA ACCTCACTAA AGGGAACAAA AGCTGGAGCT	60
CCACCGCGGT GGC GGCCGCT CTAGAGCTCG CGGCCGCGAG CTCAATTAAC CCTCACTAAA	120
GGGAGTCGAC TCGATCAGAC TGTTACTGTG TCTATGTAGA AAGAAGTAGA CATAAGAGAT	180
TCCATTTTGT TCTGTACTAA GAAAAATTCT TCTGCCTTGA GATGCTGTTA ATCTGTAACC	240
CTAGCCCCAA CCCTGTGCTC ACAGAGACAT GTGCTGTGTT GACTCAAGGT TCAATGGATT	300
TAGGGCTATG CTTTGTTAAA AAAGTGCTTG AAGATAATAT GCTTGTTAAA AGTCATCACC	360
ATTCTCTAAT CTCAAGTACC CAGGGACACA ATACACTGCG GAAGGCCGCA GGGACCTCTG	420
TCTAGGAAAG CCAGGTATTG TCCAAGATTT CTCCCATGT GATAGCCTGA GATATGGCCT	480
CATGGGAAGG GTAAGACCTG ACTGTCCCC AGCCCGACAT CCCCAGCCC GACATCCCCC	540
AGCCCGACAC CCGAAAAGGG TCTGTGCTGA GGAAGATTAN TAAAAGAGGA AGGCTCTTTG	600
CATTGAAGTA AGAAGAAGGC TCTGTCTCCT GCTCGTCCCT GGGCAATAAA ATGTCTTGGT	660
GTAAACCCG AATGTATGTT CTA CT TACTG AGAATAGGAG AAAACATCCT TAGGGCTGGA	720
GGTGAGACAC CCTGGCGGCA TACTGCTCTT TAATGCACGA GATGTTTGTN TAATTGCCAT	780
CCAGGGCCAN CCCCTTTCCT TAACTTTTTA TGAACAAAA ACTTTGTTCN CTTTTCCTGC	840
GAACCTCTCC CCCTATTANC CTATTGGCCT GCCCATCCCC TCCCCAAANG GTGAAAANAT	900
GTTCNTAAAT NCGAGGGAAT CCAAAACNTT TTCCCGTTGG TCCCCTTTC AACCCCGTCC	960
CTGGGCCNNT TTCTCCCCA ACNTGTCCCG GNTCCTTCNT TCCNCCCCC TTCCNGANA	1020

AAAAACCCCG TNTGANGGNG CCCCTCAAA TTATAACCTT TCCNAAACAA ANNGGTTTCA	1080
AGGTGGTTTG NTTCCGGTGC GGCTGGCCTT GAGGTCCCC CTNCACCCCA ATTTGGAANC	1140
CNGTTTTTTT TATTGCCCN TCCCC	1165

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1177 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

NCCNTTTAGA TGTTGACAAN NTAAACAAGC NGCTCAGGCA GCTGAAAAA GCCACTGATA	60
AAGCATCCTG GAGTATCAGA GTTTACTGTT AGATCAGCCT CATTTGACTT CCCCTCCCAC	120
ATGGTGTTTA AATCCAGCTA CACTACTTCC TGA CTCAAAC TCCACTATTC CTGTTCATGA	180
CTGTCAGGAA CTGTTGAAA CTACTGAAAC TGGCCGACCT GATCTTCAA ATGTGCCCCT	240
AGGAAAGGTG GATGCCACCG TGTTACACAGA CAGTACCNCC TTCCTCGAGA AGGGACTACG	300
AGGGGCCGGT GCANCTGTTA CCAAGGAGAC TNATGTGTTG TGGGCTCAGG CTTTACCANC	360
AAACACCTCA NCNCNNAAGG CTGAATTGAT CGCCCTCACT CAGGCTCTCG GATGGGGTAA	420
GGGATATTAA CGTTAACT GACAGCAGGT ACGCCTTTC TACTGTGCAT GTACGTGGAG	480

CCATCTACCA GGAGCGTGGG CTACTCACTC GGCAGGTGGC TGTNATCCAC TGTAANGGA	540
CATCAAAAGG AAAACNNGGC TGTGCCCCGT GGTAACCANA AANCTGATCN NCAGCTCNAA	600
GATGCTGTGT TGACTTTCAC TCNCNCCTCT TAACTTGCT GCCCACANTC TCCTTCCCA	660
ACCAGATCTG CCTGACAATC CCCATACTCA AAAAAAAAAAN AANACTGGCC CCGAACCNA	720
ACCAATAAAA ACGGGGANGG TNGGTNGANC NNCCTGACCC AAAAATAATG GATCCCCCGG	780
GCTGCAGGAA TTCAATTCAN CTTATCNAT ACCCCCAACN NGGNGGGGGG GGCCNGTNCC	840
CATTNCCCCT NTATTNATTC TTTNNCCCCC CCCCCGGCNT CCTTTTNA A CTCGTGAAAG	900
GGAAAACCTG NCTTACCAAN TTATCNCCTG GACCNCCCC TTCCNCGGTN GNTTANAAAA	960
AAAAGCCNC ANTCCNTCC NAAATTGCA CNGAAAGGNA AGGAATTTAA CCTTTATTTT	1020
TTNNTCCTTT ANTTGTNNN CCCCTTTTA CCCAGGCGAA CNGCCATCNT TTAANAAAAA	1080
AAANAGAANG TTTATTTTTC CTNGAACCA TCCAATANA AANCACCCGC NGGGGAACGG	1140
GGNGGNAGGC CNCTACCCC CTTTNTGTNG GNGGGNC	1177

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1146 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

NCCNNTTNTT GATGTTGTCT TTTTGGCCTC TCTTTGGATA CTTTCCCTCT CTCAGAGGT	60
GAAAAGGGTC AAAAGGAGCT GTTGACAGTC ATCCCAGGTG GGCCAATGTG TCCAGAGTAC	120
AGACTCCATC AGTGAGGTCA AAGCCTGGGG CTTTTCAGAG AAGGGAGGAT TATGGGTTTT	180
CCAATTATAC AAGTCAGAAG TAGAAAGAAG GGACATAAAC CAGGAAGGGG GTGGAGCACT	240
CATCACCCAG AGGGACTTGT GCCTCTCTCA GTGGTAGTAG AGGGGCTACT TCCTCCCACC	300
ACGGTTGCAA CCAAGAGGCA ATGGGTGATG AGCCTACAGG GGACATANCC GAGGAGACAT	360
GGGATGACCC TAAGGGAGTA GGCTGGTTTT AAGGCGGTGG GACTGGGTGA GGGAACTCT	420
CCTCTTCTTC AGAGAGAAGC AGTACAGGGC GAGCTGAACC GGCTGAAGGT CGAGGCGAAA	480
ACACGGTCTG GCTCAGGAAG ACCTTGGAAG TAAAATTATG AATGGTGCAT GAATGGAGCC	540
ATGGAAGGGG TGCTCCTGAC CAAACTCAGC CATTGATCAA TGTTAGGGAA ACTGATCAGG	600
GAAGCCGGGA ATTTCAATTA CAACCCGCCA CACAGCTTGA ACATTGTGAG GTTCAGTGAC	660
CCTTCAAGGG GCCACTCCAC TCCAACTTTG GCCATTCTAC TTTGCNAAAT TTCCAAAAC	720
TCCTTTTTTA AGGCCGAATC CNTANTCCCT NAAAAACNAA AAAAAATCTG CNCCTATTCT	780
GGAAAAGGCC CANCCCTTAC CAGGCTGGAA GAAATTTTNC CTTTTTTTTT TTTTGAAGG	840
CNTTTNTTAA ATTGAACCTN AATTCNCCCC CCCAAAAAAA AACCCNCCNG GGGGGCGGAT	900
TTCCAAAAAC NAATTCCCTT ACCAAAAAAC AAAAACCCNC CCTTNTTCCC TTCCNCCCTN	960
TTCTTTTAAT TAGGGAGAGA TNAAGCCCC CAATTTCCNG GNCTNGATNN GTTTCCCCC	1020

CCCCCATTTT CCNAACTTT TTCCCANCA GGAANCCNCC CTTTTTTTNG GTCNGATTNA	1080
NCAACCTTCC AAACCATTTT TCCNAAAAA NTTTGNTNGG NGGGAAAAAN ACCTNNTTTT	1140
ATAGAN	1146

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 545 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTTCATTGGG TACGGGCCCC CTCGACCTCG ACGGTATCGA TAAGCTTGAT ATCGAATTCC	60
TGCAGCCCGG GGGATCCACT AGTTCTAGAG TCAGGAAGAA CCACCAACCT TCCTGATTTT	120
TATTGGCTCT GAGTTCTGAG GCCAGTTTTC TTCTTCTGTT GAGTATGCGG GATTGTCAGG	180
CAGATCTGGC TGTGGAAAGG AGACTGTGGG CAGCAAGTTT AGAGGCGTGA CTGAAAGTCA	240
CACTGCATCT TGAGCTGCTG AATCAGCTTT CTGGTTACCA CGGGCAACAG CCGTGTTTTT	300
CTTTTGATGT CCTTTACAGT GGATTACAGC CACCTGCTGA GGTGAGTAGC CCACGCTCCT	360
GGTAGATGGC TCCACGTACA TGCACAGTAG CAAAGGCGTA CCTGCTGTCA GTGTAAACGT	420
TAATATCCTT ACCCCATCGG AGAGCCTGAG TGAGGGCGAT CAATTCAGCC CTTTGTGCT	480

GAGGTGTTTG CTGGTTAAGC CCTGAACCCA CAACACATCT GTCTCCATGG TAACAGCTGC 540
ACCGG 545

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9388 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTCGCGGCC GCGAGCTCAA TTAACCCTCA CTAAAGGGAG TCGACTCGAT CAGACTGTTA 60
CTGTGTCTAT GTAGAAAGAA GTAGACATAA GAGATTCCAT TTTGTTCTGT ACTAAGAAAA 120
ATTCTTCTGC CTTGAGATGC TGTTAATCTG TAACCCTAGC CCCAACCCTG TGCTCACAGA 180
GACATGTGCT GTGTTGACTC AAGGTTCAAT GGATTTAGGG CTATGCTTTG TTAAAAAAGT 240
GCTTGAAGAT AATATGCTTG TTAAAAGTCA TCACCATTCT CTAATCTCAA GTACCCAGGG 300
ACACAATACA CTGCGGAAGG CCGCAGGGAC CTCTGTCTAG GAAAGCCAGG TATTGTCCAA 360
GATTTCTCCC CATGTGATAG CCTGAGATAT GGCCTCATGG GAAGGGTAAG ACCTGACTGT 420
CCCCCAGCCC GACATCCCCC AGCCCGACAT CCCCAGCCC GACACCCGAA AAGGGTCTGT 480
GCTGAGGAGG ATTAGTAAAA GAGGAAGGCC TCTTTGCAGT TGAGGTAAGA GGAAGGCATC 540
TGCTCCTGCG TCGTCCCTGG GCAATAGAAT GTCTTGGTGT AAAACCCGAT TGTATGTTCT 600

ACTTACTGAG ATAGGAGAAA ACATCCTTAG GGCTGGAGGT GAGACACGCT GCGGCAATA	660
CTGCTCTTTA ATGCACCGAG ATGTTTGTAT AAGTGCACAT CAAGGCACAG CACCTTTCCT	720
TAAACTTATT TATGACACAG AGACCTTTGT TCACGTTTTC CTGCTGACCC TCTCCCCACT	780
ATTACCCTAT TGGCCTGCCA CATCCCCCTC TCCGAGATGG TAGAGATAAT GATCAATAAA	840
TACTGAGGGA ACTCAGAGAC CAGTGTCCCT GTAGGTCCTC CGTGTGCTGA GCGCCGGTCC	900
CTTGGGCTCA CTTTTCTTTC TCTATACTTT GTCTCTGTGT CTCTTCTTT TCTCAGTCTC	960
TCGTTCCACC TGACGAGAAA TACCCACAGG TGTGGAGGGG CAGGCCACCC CTTCAATAAT	1020
TTACTAGCCT GTTCGCTGAC AACAAGACTG GTGGTGCAGA AGGTTGGGTC TTGGTGTTC	1080
CCGGGTGGCA GGCATGGGCC AGGTGGGAGG GTCTCCAGCG CCTGGTGCAA ATCTCCAAGA	1140
AAGTGCAGGA AACAGCACCA AGGGTGATTG TAAATTTTGA TTTGGCGCGG CAGGTAGCCA	1200
TTCCAGCGCA AAAATGCGCA GGAAAGCTTT TGCTGTGCTT GTAGGCAGGT AGGCCCCAAG	1260
CACTTCTTAT TGGCTAATGT GGAGGGAACC TGCACATCCA TTGGCTGAAA TCTCCGTCTA	1320
TTTGAGGCTG ACTGAGCGCG TTCCTTCTT CTGTGTTGCC TGGAAACGGA CTGTCTGCCT	1380
AGTAACATCT GATCACGTTT CCCATTGGCC GCCGTTTCCG GAAGCCCGCC CTCCCATTTT	1440
CGGAAGCCTG GCGCAAGGTT GGTCTGCAGG TGGCCTCCAG GTGCAAAGTG GGAAGTGTGA	1500
GTCCTCAGTC TTGGGCTATT CGGCCACGTG CCTGCCGGAC ATGGGACGCT GGAGGGTCAG	1560
CAGCGTGGAG TCCTGGCCTT TTGCGTCCAC GGGTGGGAAA TTGGCCATTG CCACGGCGGG	1620
AACTGGGACT CAGGCTGCCC CCCGGCCGTT TCTCATCCGT CCACCGGACT CGTGGGCGCT	1680

CGCACTGGCG CTGATGTAGT TTCCTGACCT CTGACCCGTA TTGTCTCCAG ATTAAAGGTA	1740
AAAACGGGGC TTTTTCAGCC CACTCGGGTA AAACGCCTTT TGATTCTAG GCAGGTGTTT	1800
TGTTGCACGC CTGGGAGGGA GTGACCCGCA GGTGAGGTT TATTAAAATA CATTCCTGGT	1860
TTATGTTATG TTTATAATAA AGCACCCCAA CCTTTACAAA ATCTCACTTT TTGCCAGTTG	1920
TATTATTTAG TGGACTGTCT CTGATAAGGA CAGCCAGTTA AAATGGAATT TTGTTGTTGC	1980
TAATTAAACC AATTTTtagT TTTGGTGTtt GTCCTAATAG CAACAACttC TCAGGCTTTA	2040
TAAAACCATA TTTCTTGGGG GAAATTTCTG TGTAAGGCAC AGCGAGTTAG TTTGGAATTG	2100
TTTTAAAGGA AGTAAGTCC TGGTTTTGAT ATCTTAGTAG TGTAATGCCC AACCTGGTTT	2160
TTACTAACCC TGTTTTTAGA CTCTCCCTTT CCTTAAATCA CCTAGCCTTG TTTCCACCTG	2220
AATTGACTCT CCCTTAGCTA AGAGCGCCAG ATGGACTCCA TCTTGGCTCT TTCACTGGCA	2280
GCCCCttCCT CAAGGACTTA ACTTGTGCAA GCTGACTCCC AGCACATCCA AGAATGCAAT	2340
TAACTGTtAA GATACTGTGG CAAGCTATAT CCGCAGTTCC GAGGAATTCA TCCGATTGAT	2400
TATGCCCAAA AGCCCCGCGT CTATCACCTT GTAATAATCT TAAAGCCCTT GCACCTGGAA	2460
CTATTAACtt TCCTGTAAcc ATTTATCCTT TTAACttttT TGCTTACTtt ATTTCTGTAA	2520
AATTGttttA ACTAGACCTC CCCTCCCTT TCTAAACCAa AGTATAAAAG AAGATCTAGC	2580
CCCTTCTTCA GAGCGGAGAG AATTTTGAGC ATTAGCCATC TCTTGGCGGC CAGCTAAATA	2640
AATGGACTTT TAATTTGTCT CAAAGTGTGG CGTTTTCTCT AACTCGCTCA GGTACGACAT	2700
TTGGAGGCCC CAGCGAGAAA CGTCACCGGG AGAAACGTCA CCGGGCGAGA GCCGGGCCCG	2760

CTGTGTGCTC CCCCGBAAGG ACAGCCAGCT TGTAGGGGGG AGTGCCACCT GAAAAAAAAA	2820
TTTCCAGGTC CCCAAAGGGT GACCGTCTTC CGGAGGACAG CGGATCGACT ACCATGCGGG	2880
TGCCCACCAA AATTCCACCT CTGAGTCCTC AACTGCTGAC CCCGGGGTCA GGTAGGTCAG	2940
ATTTGACTTT GGTCTGGCA GAGGGAAGCG ACCCTGATGA GGGTGTCCCT CTTTGGACTC	3000
TGCCCATTTC TCTAGGATGC TAGAGGGTAG AGCCCTGGTT TTCTGTTAGA CGCCTCTGTG	3060
TCTCTGTCTG GGAGGGAAGT GGCCCTGACA GGGGCCATCC CTTGAGTCAG TCCACATCCC	3120
AGGATGCTGG GGGACTGAGT CCTGGTTTCT GGCAGACTGG TCTCTCTCTC TCTCTTTTTC	3180
TATCTCTAAT CTTTCCTTGT TCAGGTTTCT TGGAGAATCT CTGGGAAAGA AAAAAGAAAA	3240
ACTGTTATAA ACTCTGTGTG AATGGTGAAT GAATGGGGGA GGACAAGGGC TTGCGCTTGT	3300
CCTCCAGTTT GTAGCTCCAC GGCAGAAAGCT ACGGAGTTCA AGTGGGCCCT CACCTGCGGT	3360
TCCGTGGCGA CCTCATAAGG CTTAAGGCAG CATCCGGCAT AGCTCGATCC GAGCCGGGGG	3420
TTTATACCGG CCTGTCAATG CTAAGAGGAG CCCAAGTCCC CTAAGGGGGA GCGGCCAGGC	3480
GGGCATCTGA CTGATCCCAT CACGGGACCC CCTCCCCTTG TTTGTCTAAA AAAAAAAAAA	3540
GAAGAACTG TCATAACTGT TTACATGCCC TAGGGTCAAC TGTTTGTTTT ATGTTTATTG	3600
TTCTGTTGGG TGTCTATTGT CTGTTTAGT GGTGTGCAAG GTTTTGCATG TCAGGACGTC	3660
GATATTGCCC AAGACGCTG GGTAAGAACT TCTGCAAGGT CCTAGTGCT GATTTTTTGT	3720
CACAGGAGGT TAAATTTCTC ATCAATCATT TAGGCTGGCC ACCACAGTCC TGTCTTTTCT	3780
GCCAGAAGCA AGTCAGGTGT TGTTACGGGA ATGAGTGTA AAAAAATTTC GCCTGATTGG	3840

GATTTCTGGC ACCATGATGG TTGTATTTAG ATTGTCATAC CCCACATCCA GGTGATTGG	3900
ACCTCCTCTA AACTAAACTG GTGGTGGGT CAAAACAGCC ACCCTGCAGA TTCCTTGCT	3960
CACCTCTTTG GTCATTCTGT AACTTTTCCT GTGCCCTTAA ATAGCACACT GTGTAGGGAA	4020
ACCTACCCTC GTACTGCTTT ACTTCGTTTA GATTCTTACT CTGTTCTCT GTGGCTACTC	4080
TCCCATCTTA AAAACGATCC AAGTGGTCCT TTCCTCCTC CTGCCCCCT ACCCCACACA	4140
TCTCGTTTTC CAGTGCGACA GCAAGTTCAG CGTCTCCAGG ACTTGGCTCT GCTCTCACTC	4200
CTTGAACCCT TAAAAGAAAA AGCTGGGTTT GAGCTATTTG CCTTTGAGTC ATGGAGACAC	4260
AAAAGGTATT TAGGGTACAG ATCTAGAAGA AGAGAGAGAA CACCTAGATC CAACTGACCC	4320
AGGAGATCTC GGGCTGGCCT CTAGTCCTCC TCCCTCAATC TTAAAGCTAC AGTGATGTGG	4380
CAAGTGGTAT TTAGCTGTTG TGGTTTTTCT GCTCTTCTG GTCATGTTGA TTCTGTTCTT	4440
TCGATACTCC AGCCCCCAG GGAGTGAGTT TCTCTGTCTG TGCTGGGTTT GATATCTATG	4500
TTCAAATCTT ATTAAATTGC CTTCAAAAAA AAAAAAAAAA GGGAAACACT TCCTCCCAGC	4560
CTTGTAAGGG TTGGAGCCCT CTCCAGTATA TGCTGCAGAA TTTTCTCTC GGTTTCTCAG	4620
AGGATTATGG AGTCCGCCTT AAAAAAGGCA AGCTCTGGAC ACTCTGCAA GTAGAATGGC	4680
CAAAGTTTGG AGTTGAGTGG CCCCTTGAAG GGTCAGTAA CCTCACAATT GTTCAAGCTG	4740
TGTGGCGGGT TGTTACTGAA ACTCCCGGCC TCCCTGATCA GTTTCCTAC ATTGATCAAT	4800
GGCTGAGTTT GGTGAGGAGC ACCCCTTCCA TGGCTCCACT CATGCACCAT TCATAATTTT	4860
ACCTCAAGG TCCTCCTGAG CCAGACCGTG TTTTCGCTC GACCCTCAGC CGGTTGAGCT	4920

CGCCCTGTAC TGCCTCTCTC TGAAGAAGAG GAGAGTCTCC CTCACCCAGT CCCACCGCCT 4980

TAAAACCAGC CTA CTCTCCCTT AGGGTCATCC CATGTCTCCT CGGCTATGTC CCCTGTAGGC 5040

TCATCACCCA TTGCCTCTTG GTTGCAACCG TGGTGGGAGG AAGTAGCCCC TCTACTACCA 5100

CTGAGAGAGG CACAAGTCCC TCTGGGTGAT GAGTGCTCCA CCCCCTTCCT GGTTTATGTC 5160

CCTTCTTTCT ACTTCTGACT TGTATAATTG GAAAACCCAT AATCCTCCCT TCTCTGAAAA 5220

GCCCCAGGCT TTGACCTCAC TGATGGAGTC TGTACTCTGG ACACATTGGC CCACCTGGGA 5280

TGACTGTCAA CAGCTCCTTT TGACCCCTTT CACCTCTGAA GAGAGGGAAA GTATCCAAAG 5340

AGAGGCCAAA AAGTACAACC TCACATCAAC CAATAGGCCG GAGGAGGAAG CTAGAGGAAT 5400

AGTGATTAGA GACCCAATTG GGACCTAATT GGGACCCAAA TTTCTCAAGT GGAGGGAGAA 5460

CTTTTGACGA TTTCCACCGG TATCTCCTCG TGGGTATTCA GGGAGCTGCT CAGAAACCTA 5520

TAAACTTGTC TAAGGCGACT GAAGTCGTCC AGGGGCATGA TGAGTCACCA GGAGTGTTTT 5580

TAGAGCACCT CCAGGAGGCT TATCGGATTT ACACCCCTTT TGACCTGGCA GCCCCGAAA 5640

ATAGCCATGC TCTTAATTTG GCATTTGTGG CTCAGGCAGC CCCAGATAGT AAAAGGAAAC 5700

TCCAAAAACT AGAGGGATTT TGCTGGAATG AATACCAGTC AGCTTTTAGA GATAGCCTAA 5760

AAGGTTTTTG ACAGTCAAGA GGTTGAAAAA CAAAAACAAG CAGCTCAGGC AGCTGAAAAA 5820

AGCCACTGAT AAAGCATCCT GGAGTATCAG AGTTTACTGT TAGATCAGCC TCATTTGACT 5880

TCCCCTCCA CATGGTGTTT AAATCCAGCT ACACTACTTC CTGACTCAA CTCCACTATT 5940

CCTGTTTATG ACTGTCAGGA ACTGTTGGAA ACTACTGAAA CTGGCCGACC TGATCTTCAA 6000

AATGTGCCCC TAGGAAAGGT GGATGCCACC GTG TTCACAG ACAGTAGCAG CTCCTCGAG	6060
AAGGGACTAC GAAAGGCCGG TGCAGCTGTT ACCATGGAGA CAGATGTGTT GTGGGCTCAG	6120
GCTTTACCAG CAAACACCTC AGCACAAAAG GCTGAATTGA TCGCCCTCAC TCAGGCTCTC	6180
CGATGGGGTA AGGATATTAA CGTTAACT GACAGCAGGT ACGCCTTTGC TACTGTGCAT	6240
GTACGTGGAG CCATCTACCA GGAGCGTGGG CTA CTACCT CAGCAGGTGG CTGTAATCCA	6300
CTGTAAAGGA CATCAAAGG AAAACACGGC TGTGCCCCGT GGTAACCAGA AAGCTGATTC	6360
AGCAGCTCAA GATGCAGTGT GACTTTCAGT CACGCCTCTA AACTTGCTGC CCACAGTCTC	6420
CTTCCACAG CCAGATCTGC CTGACAATCC CGCATACTCA ACAGAAGAAG AAAACTGGCC	6480
TCAGAACTCA GAGCCAATAA AAATCAGGAA GGTGGTGGGA TTCTTCCTGA CTCTAGAATC	6540
TTCATACCCC GAACTCTTGG GAAACTTTA ATCAGTCACC TACAGTCTAC CACCCATTTA	6600
GGAGGAGCAA AGCTACCTCA GCTCCTCCGG AGCCGTTTTA AGATCCCCCA TCTTCAAAGC	6660
CTAACAGATC AAGCAGCTCT CCGGTGCACA ACCTGCGCCC AGGTAAATGC CAAAAAAGGT	6720
CCTAAACCCA GCCCAGGCCA CCGTCTCAA GAAACTCAC CAGGAGAAAA GTGGGAAATT	6780
GACTTTACAG AAGTAAAACC ACACCGGGCT GGGTACAAAT ACCTTCTAGT ACTGGTAGAC	6840
ACCTTCTCTG GATGGACTGA AGCATTTGCT ACCAAAAACG AACTGTCAA TATGGTAGTT	6900
AAGTTTTTAC TCAATGAAAT CATCCCTCGA CGTGGGCTGC CTGTTGCCAT AGGGTCTGAT	6960
AATGGACCGG CCTTCGCCTT GTCTATAGTT TAGTCAGTCA GTAAGGCGTT AAACATTCAA	7020
TGGAAGCTCC ATTGTGCCTA TCGACCCAG AGCTCTGGGC AAGTAGAACG CATGAACTGC	7080

ACCCTAAAAA	ACACTCTTAC	AAAATTAATC	TTAGAAACCG	GTGTAAATTG	TGTAAGTCTC	7140
CTTCCTTTAG	CCCTACTTAG	AGTAAGGTGC	ACCCCTTACT	GGGCTGGGTT	CTTACCTTTT	7200
GAAATCATGT	ATGGGAGGGC	GCTGCCTATC	TTGCCTAAGC	TAAGAGATGC	CCAATTGGCA	7260
AAAATATCAC	AACTAATTT	ATTACAGTAC	CTACAGTCTC	CCCAACAGGT	ACAAGATATC	7320
ATCCTGCCAC	TTGTTGAGG	AACCCATCCC	AATCCAATTC	CTGAACAGAC	AGGGCCCTGC	7380
CATTCATTCC	CGCCAGGTGA	CCTGTTGTTT	GTAAAAAGT	TCCAGAGAGA	AGGACTCCCT	7440
CCTGCTTGGA	AGAGACCTCA	CACCGTCATC	ACGATGCCAA	CGGCTCTGAA	GGTGGATGGC	7500
ATTCCTGCGT	GGATTCATCA	CTCCCGCATC	AAAAAGGCCA	ACGGAGCCCA	ACTAGAAACA	7560
TGGGTCCCCA	GGGCTGGGTC	AGGCCCTTA	AACTGCACC	TAAGTTGGGT	GAAGCCATTA	7620
GATTAATTCT	TTTTCTTAAT	TTGTAAAAC	AATGCATAGC	TTCTGTCAA	CTTATGTATC	7680
TTAAGACTCA	ATATAACCCC	CTTGTTATAA	CTGAGGAATC	AATGATTGGA	TTCCCCAAAA	7740
ACACAAGTGG	GGAATGTAGT	GTCCAACCTG	GTTTTTACTA	ACCCTGTTTT	TAGACTCTCC	7800
CTTTCCTTTA	ATCACTCAGC	CTTGTTTCCA	CCTGAATTGA	CTCTCCCTTA	GCTAAGAGCG	7860
CCAGATGGAC	TCCATCTTGG	CTCTTTCACT	GGCAGCCGCT	TCCTCAAGGA	CTTAAGTTGT	7920
GCAAGCTGAC	TCCCAGCACA	TCCAAGAATG	CAATTAAGTG	ATAAGATACT	GTGGCAAGCT	7980
ATATCCGCAG	TTCCAGGAA	TTGTTCCAAT	TGATTACACC	CAAAGCCCC	GCGTCTATCA	8040
CCTTGTAATA	ATCTTAAAGC	CCCTGCACCT	GGAAGTATTA	ACGTTCTGT	AACCATTTAT	8100
CCTTTTAACT	TTTTTGCTA	CTTTATTCT	GTAAAATTGT	TTAACTAGA	CCCCCCTCT	8160

CCTTTCTAAA CCAAAGTATA AAAGCAAATC TAGCCCCTTC TTCAGGCCGA GAGAATTTCTG	8220
AGCGTTAGCC GTCTCTTGGC CACCAGCTAA ATAAACGGAT TCTTCATGTG TCTCAAAGTG	8280
TGGCGTTTTTC TCTAACTCGC TCAGGTACGA CCGTGGTAGT ATTTTCCCCA ACGTCTTATT	8340
TTTAGGGCAC GTATGTAGAG TAACTTTTAT GAAAGAAACC AGTTAAGGAG GTTTTGGGAT	8400
TTCCTTTATC AACTGTAATA CTGGTTTTGA TTATTTATTT ATTTATTTAT TTTTTTTGAG	8460
AAGGAGTTTC ACTCTTGTTG CCCAGGCTGG AGTGCAATGG TCGATCTTG GCTCACTGCA	8520
ACTTCCGCCT CCCAGGTTCA AGCGATTCTC CTGCCTCAGC CTCGAGAGTA GCTGGGATTA	8580
TAGGCATGCG CCACCACACC CAGCTAATTT TGTATTTTGA GTAAAGATGG GGTTCCTTCA	8640
TGTTGGTCAA GCTGGTCTGG AACTCCCCGC CTCGGGTGAT CTGCCCCCCT CGGCCTCCGA	8700
AAGTGCTGGG ATTACAGGTG TGATCCACCA CACCCAGCCG ATTTATATGT ATATAAATCA	8760
CATTCCTCTA ACCAAAATGT AGTGTTTCCT TCCATCTTGA ATATAGGCTG TAGACCCCGT	8820
GGGTATGGGA CATTGTTAAC AGTGAGACCA CAGCAGTTTT TATGTCATCT GACAGCATCT	8880
CCAAATAGCC TTCATGGTTG TCACTGCTTC CCAAGACAAT TCCAAATAAC ACTTCCCAGT	8940
GATGACTTGC TACTTGCTAT TGTTACTTAA TGTGTTAAGG TGGCTGTTAC AGACACTATT	9000
AGTATGTCAG GAATTACACC AAAATTTAGT GGCTCAAACA ATCATTTTAT TATGTATGTG	9060
GATTCTCATG GTCAGGTCAG GATTTTCAGAC AGGGCACAAG GGTAGCCAC TTGTCTCTGT	9120
CTATGATGTC TGGCCTCAGC ACAGGAGACT CAACAGCTGG GGTCTGGGAC CATTTGGAGG	9180
CTTGTTCCCT CACATCTGAT ACCTGGCTTG GGATGTTGGA AGAGGGGGTG AGCTGAGACT	9240

GAGTGCCTAT ATGTAGTGTT TCCATATGGC CTTGACTTCC TTACAGCCTG GCAGCCTCAG	9300
GGTAGTCAGA ATTCTTAGGA GGCACAGGGC TCCAGGGCAG ATGCTGAGGG GTCTTTTATG	9360
AGGTAGCACA GCAAATCCAC CCAGGATC	9388

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3646 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGAAACACT TCCTCCCAGC CTTGTAAGGG TTGGAGCCCT CTCCAGTATA TGCTGCAGAA	60
TTTTTCTCTC GGTTTCTCAG AGGATTATGG AGTCCGCCTT AAAAAAGGCA AGCTCTGGAC	120
ACTCTGCAAA GTAGAATGGC CAAAGTTTGG AGTTGAGTGG CCCCTTGAAG GGTCAC TGAA	180
CCTCACAATT GTTCAAGCTG TGTGGCGGGT TGTTACTGAA ACTCCCGGCC TCCCTGATCA	240
GTTTCCCTAC ATTGATCAAT GGCTGAGTTT GGTCAGGAGC ACCCCTTCCG TGGCTCCACT	300
CATGCACCAT TCATAATTTT ACCTCCAAGG TCCTCCTGAG CCAGACCGTG TTTTCGCCTC	360
GACCCTCAGC CGGTTTCGGCT CGCCCTGTAC TGCCTCTCTC TGAAGAAGAG GAGAGTCTCC	420
CTCACCAGT CCCACCGCCT TAAACCAGC CTACTCCCTT AGGGTCATCC CATGTCTCCT	480
CGGCTATGTC CCCTGTAGGC TCATCACCCA TTGCCTCTTG GTTGCAACCG TGGTGGGAGG	540
AAGTAGCCCC TCTACTACCA CTGAGAGAGG CACAAGTCCC TCTGGGTGAT GAGTGCTCCA	600

CCCCCTTCCT GGTTTATGTC CCTTCTTTCT ACTTCTGACT TGTATAATTG GAAAACCCAT	660
AATCCTCCCT TCTCTGAAAA GCCCCAGGCT TTGACCTCAC TGATGGAGTC TGTACTCTGG	720
ACACATTGGC CCACCTGGGA TGACTGTCAA CAGCTCCTTT TGACCCTTTT CACCTCTGAA	780
GAGAGGGAAA GTATCCAAAG AGAGGCCAAA AAGTACAACC TCACATCAAC CAATAGGCCG	840
GAGGAGGAAG CTAGAGGAAT AGTGATTAGA GACCCAATTG GGACCTAATT GGGACCCAAA	900
TTTCTCAAGT GGAGGGAGAA CTTTTGACGA TTTCCACCGG TATCTCCTCG TGGGTATTCA	960
GGGAGCTGCT CAGAAACCTA TAAACTTGTC TAAGGCGACT GAAGTCGTCC AGGGGCATGA	1020
TGAGTCACCA GGAGTGTTTT TAGAGCACCT CCAGGAGGCT TATCAGATTT ACACCCCTTT	1080
TGACCTGGCA GCCCCGAAA ATAGCCATGC TCTTAATTTG GCATTTGTGG CTCAGGCAGC	1140
CCCAGATAGT AAAAGGAAAC TCCAAAACT AGAGGGATTT TGCTGGAATG AATACCAGTC	1200
AGCTTTTAGA GATAGCCTAA AAGGTTTTTG ACAGTCAAGA GGTGAAAAA CAAAAACAAG	1260
CAGCTCAGGC AGCTGAAAAA AGCCACTGAT AAAGCATCCT GGAGTATCAG AGTTTACTGT	1320
TAGATCAGCC TCATTTGACT TCCCCTCCCA CATGGTGTTT AAATCCAGCT ACACTACTTC	1380
CTGACTCAAA CTCCACTATT CCTGTTCATG ACTGTCAGGA ACTGTTGGAA ACTACTGAAA	1440
CTGGCCGACC TGATCTTCAA AATGTGCCCC TAGGAAAGGT GGATGCCACC ATGTTACAG	1500
ACAGTAGCAG CTTCTCGAG AAGGGACTAC GAAAGGCCGG TGCAGCTGTT ACCATGGAGA	1560
CAGATGTGTT GTGGGCTCAG GCTTTACCAG CAAACACCTC AGCACAAAAG GCTGAATTGA	1620
TCGCCCTCAC TCAGGCTCTC CGATGGGGTA AGGATATTAA CGTTAACT GACAGCAGGT	1680

ACGCCTTTGC TACTGTGCAT GTACGTGGAG CCATCTACCA GGAGCGTGGG CTACTCACCT	1740
CAGCAGGTGG CTGTAATCCA CTGTAAAGGA CATCAAAAGG AAAACACGGC TGTGCCCCGT	1800
GGTAACCAGA AAGCTGATTC AGCAGCTCAA GATGCAGTGT GACTTTCAGT CACGCCTCTA	1860
AACTTGCTGC CCACAGTCTC CTTTCCACAG CCAGATCTGC CTGACAATCC CGCATACTCA	1920
ACAGAAGAAG AAAACTGGCC TCAGAACTCA GAGCCAATAA AAATCAGGAA GGTGCGTGGG	1980
TTCTTCCTGA CTCTAGAATC TTCATACCCC GAACTCTTGG GAAAACTTTA ATCAGTCACC	2040
TACAGTCTAC CACCCATTTA GGAGGAGCAA AGCTACCTCA GCTCCTCCGG AGCCGTTTTA	2100
AGATCCCCCA TCTTCAAAGC CTAACAGATC AAGCAGCTCT CCGGTGCACA ACCTGCGCCC	2160
AGGTAAATGC CAAAAAAGGT CCTAAACCCA GCCCAGGCCA CCGTCTCCAA GAAAACTCAC	2220
CAGGAGAAAA GTGGGAAATT GACTTTACAG AAGTAAACC ACACCGGGCT GGGTACAAAT	2280
ACCTTCTAGT ACTGGTAGAC ACCTTCTCTG GATGGACTGA AGCATTGCT ACCAAAAACG	2340
AAACTGTCAA TATGGTAGTT AAGTTTTTAC TCAATGAAAT CATCCCTCGA CATGGGCTGC	2400
CTGTTTGCCA TAGGGTCTGA TAATGGACCG GCCTTCGCT TGTCTATAGT TTAGTCAGTC	2460
AGTAAGGCGT TAAACATTCA ATGGAAGCTC CATTGTGCCT ATCGACCCCA GAGCTCTGGG	2520
CAAGTAGAAC GCATGAACTG CACCCTAAAA AACACTCTTA CAAAATTAAT CTTAGAAACC	2580
GGTGTAATTT GTGTAAGTCT CCTTCCTTTA GCCCTACTTA GAGTAAGGTG CACCCCTTAC	2640
TGGGCTGGGT TCTTACCTTT TGAAATCATG TATGGGAGGG TGCTGCCTAT CTTGCCTAAG	2700
CTAAGAGATG CCCAATTGGC AAAAATATCA CAACTAATT TATTACAGTA CCTACAGTCT	2760

CCCCAACAGG TACAAGATAT CATCCTGCCA CTTGTTCGAG GAACCCATCC CAATCCAATT	2820
CCTGAACAGA CAGGGCCCTG CCATTCATTC CCGCCAGGTG ACCTGTTGTT TGTTAAAAAG	2880
TTCCAGAGAG AAGGACTCCC TCCTGCTTGG AAGAGACCTC ACACCGTCAT CACGATGCCA	2940
ACGGCTCTGA AGGTGGATGG CATTCTGCG TGGATTCATC ACTCCCGCAT CAAAAAGGCC	3000
AACAGAGCCC AACTAGAAAC ATGGGTCCCC AGGGCTGGGT CAGGCCCTT AAAACTGCAC	3060
CTAAGTTGGG TGAAGCCATT AGATTAATTC TTTTCTTAA TTTTGTA AAA CAATGCATAG	3120
CTTCTGTCAA ACTTATGTAT CTTAAGACTC AATATAACCC CCTTGTTATA ACTGAGGAAT	3180
CAATGATTTG ATTCCCCCAA AAACACAAGT GGGGAATGTA GTGTCCAACC TGGTTTTTAC	3240
TAACCCTGTT TTTAGACTCT CCCTTTCCTT TAATCACTCA GCTTGTTTCC ACCTGAATTG	3300
ACTCTCCCTT AGCTAAGAGC GCCAGATGGA CTCCATCTTG GCTCTTTCAC TGGCAGCCGC	3360
TTCTCAAGG ACTTAACTTG TGCAAGCTGA CTCCCAGCAC ATCCAAGAAT GCAATTA ACT	3420
GATAAGATAC TGTGGCAAGC TATATCCGCA GTTCCCAGGA ATTCGTCCAA TTGATCACAG	3480
CCCCTCTACC CTTAGCAAC CACCACCCTG ATCAGTCAGC AGCCATCAGC ACCGAGGCAA	3540
GGCCCTCCAC CAGCAAAAAG ATTCTGACTC ACTGAAGACT TGGATGATCA TTAGTATTTT	3600
TAGCAGTAAA GTTTTTTTTT CTTTTCTTT CTTTTTTCT CGTGCC	3646

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCTCAACCTC

10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGGCTATTT TCGGGGGCTG ACA

23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCGGTATCTC CTCGTGGGTA TT

22

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTTCAACCTC

10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGCCTGAGC CACAAATG

18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCGGAGGAGG AAGCTAGAGG AATA

24

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTTTTTTTT TTAG

14

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ser Ser Gly Gly Arg Thr Phe Asp Asp Phe His Arg Tyr Leu Leu Val
1 5 10 15

Gly Ile

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gln Gly Ala Ala Gln Lys Pro Ile Asn Leu Ser Lys Xaa Ile Glu Val
1 5 10 15

Val Gln Gly His Asp Glu

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ser Pro Gly Val Phe Leu Glu His Leu Gln Glu Ala Tyr Arg Ile Tyr
1 5 10 15

Thr Pro Phe Asp Leu Ser Ala
20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Tyr Leu Leu Val Gly Ile Gln Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Ala Ala Gln Lys Pro Ile Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Asn Leu Ser Lys Xaa Ile Glu Val Val
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Val Val Gln Gly His Asp Glu Ser
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

His Leu Gln Glu Ala Tyr Arg Ile Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Asn Leu Ala Phe Val Ala Gln Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Phe Val Ala Gln Ala Ala Pro Asp Ser
1 5

Claims

1. An isolated DNA molecule, comprising:
 - (a) a human endogenous retroviral sequence, wherein said retroviral sequence is preferentially expressed in a tumor tissue;
 - (b) a variant of said human endogenous retroviral sequence that contains one or more nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% of the nucleotide positions, such that the antigenic and/or immunogenic properties of the polypeptide encoded by the human endogenous retroviral sequence are retained; or
 - (c) a nucleotide sequence encoding an epitope of a polypeptide encoded by at least one of the above sequences.
2. An isolated DNA molecule encoding an epitope of a polypeptide, wherein said polypeptide is encoded by:
 - (a) a nucleotide sequence transcribed from the sequence of SEQ ID NO:11; or
 - (b) a variant of said nucleotide sequence that contains one or more nucleotide substitutions, deletions, insertions and/or modifications at no more than 20% of the nucleotide positions, such that the antigenic and/or immunogenic properties of the polypeptide encoded by the nucleotide sequence are retained.
3. A recombinant expression vector comprising a DNA molecule according to claim 1 or claim 2.
4. A host cell transformed or transfected with an expression vector according to claim 3.
5. A polypeptide comprising an amino acid sequence encoded by a DNA molecule according to claim 1 or claim 2.
6. A monoclonal antibody that binds to a polypeptide according to claim 5.
7. A method for determining the presence of a cancer in a patient comprising detecting, within a biological sample obtained from a patient, a polypeptide according to claim 5, and therefrom determining the presence of cancer in the patient.

8. The method of claim 7 wherein the biological sample is a tumor sample.
9. The method of claim 7 wherein the step of detecting comprises contacting the biological sample with a monoclonal antibody according to claim 6.
10. The method of claim 7 wherein the polypeptide comprises an amino acid sequence encoded by a human endogenous retroviral sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 - SEQ ID NO:10 and SEQ ID NO:12.
11. A method for determining the presence of a cancer in a patient comprising detecting, within a biological sample obtained from a patient, an RNA molecule encoding a polypeptide according to claim 5, and therefrom determining the presence of cancer in the patient.
12. The method of claim 11 wherein the biological sample is a tumor sample.
13. The method of claim 11 wherein the step of detecting comprises:
 - (a) preparing cDNA from RNA molecules within the biological sample; and
 - (b) specifically amplifying cDNA molecules that are capable of encoding at least a portion of a polypeptide according to claim 5.
14. The method of claim 11 wherein the polypeptide comprises an amino acid sequence encoded by a human endogenous retroviral sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 - SEQ ID NO:10 and SEQ ID NO:12.
15. A polypeptide according to claim 5 for use within a method for detecting the presence of a cancer in a patient.
16. The polypeptide of claim 15 wherein the polypeptide comprises an amino acid sequence encoded by a human endogenous retroviral sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 - SEQ ID NO:10 and SEQ ID NO:12.
17. A method for monitoring the progression of a cancer in a patient, comprising:

- (a) detecting an amount, in a biological sample obtained from a patient, of a polypeptide according to claim 5;
- (b) subsequently repeating step (a); and
- (c) comparing the amounts of polypeptide detected in steps (a) and (b), and therefrom monitoring the progression of cancer in the patient.

18. The method of claim 17 wherein the biological sample is a tumor sample.

19. The method of claim 17 wherein the step of detecting comprises contacting a portion of the biological sample with a monoclonal antibody according to claim 6.

20. The method of claim 17 wherein the polypeptide comprises an amino acid sequence encoded by a human endogenous retroviral sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 - SEQ ID NO:10 and SEQ ID NO:12.

21. A method for monitoring the progression of a cancer in a patient, comprising:

- (a) detecting an amount, within a biological sample obtained from a patient, of an RNA molecule encoding a polypeptide according to claim 5;
- (b) subsequently repeating step (a); and
- (c) comparing the amounts of RNA molecules detected in steps (a) and (b), and therefrom monitoring the progression of cancer in the patient.

22. The method of claim 21 wherein the step of detecting comprises:

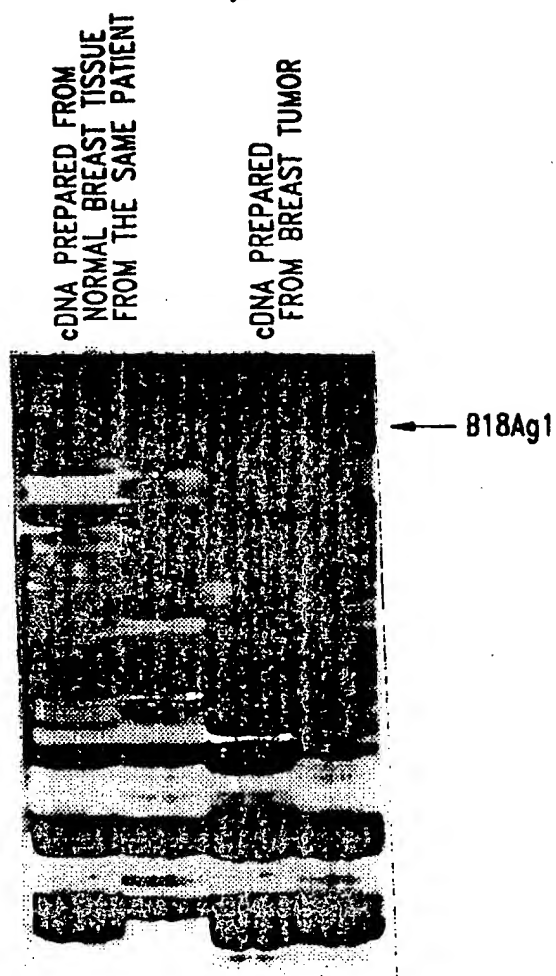
- (a) preparing cDNA from RNA molecules within the biological sample;
- and
- (b) specifically amplifying cDNA molecules that are capable of encoding at least a portion of a polypeptide according to claim 5.

23. The method of claim 21 wherein the polypeptide comprises an amino acid sequence encoded by a human endogenous retroviral sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 - SEQ ID NO:10 and SEQ ID NO:12.

24. A pharmaceutical composition, comprising:

- (a) a polypeptide according to claim 5; and

- (b) a physiologically acceptable carrier.
25. A vaccine, comprising:
- (a) a polypeptide according to claim 5; and
 - (b) an immune response enhancer.
26. A diagnostic kit comprising:
- (a) one or more monoclonal antibodies according to claim 6; and
 - (b) a detection reagent.
27. The kit of claim 26 wherein the monoclonal antibody(s) are immobilized on a solid support.
28. A diagnostic kit comprising a first polymerase chain reaction primer and a second polymerase chain reaction primer, the first and second primers each comprising at least about 10 contiguous nucleotides of an RNA molecule encoding a polypeptide according to claim 5.
29. A diagnostic kit comprising at least one oligonucleotide probe, the oligonucleotide probe comprising at least about 15 contiguous nucleotides of a DNA molecule according to claim 1 or claim 2.
-

*Fig. 1*

2/6

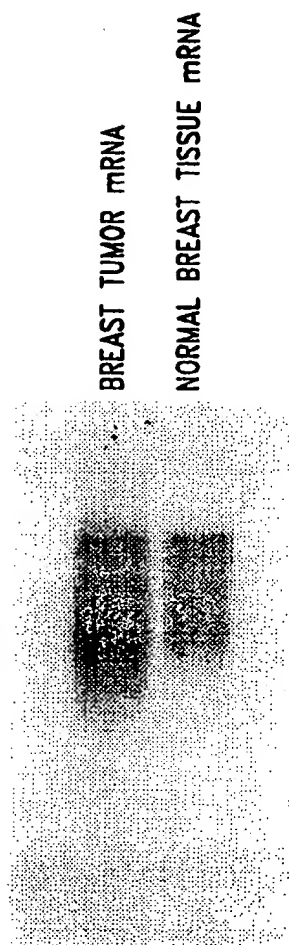


Fig. 2

3/6

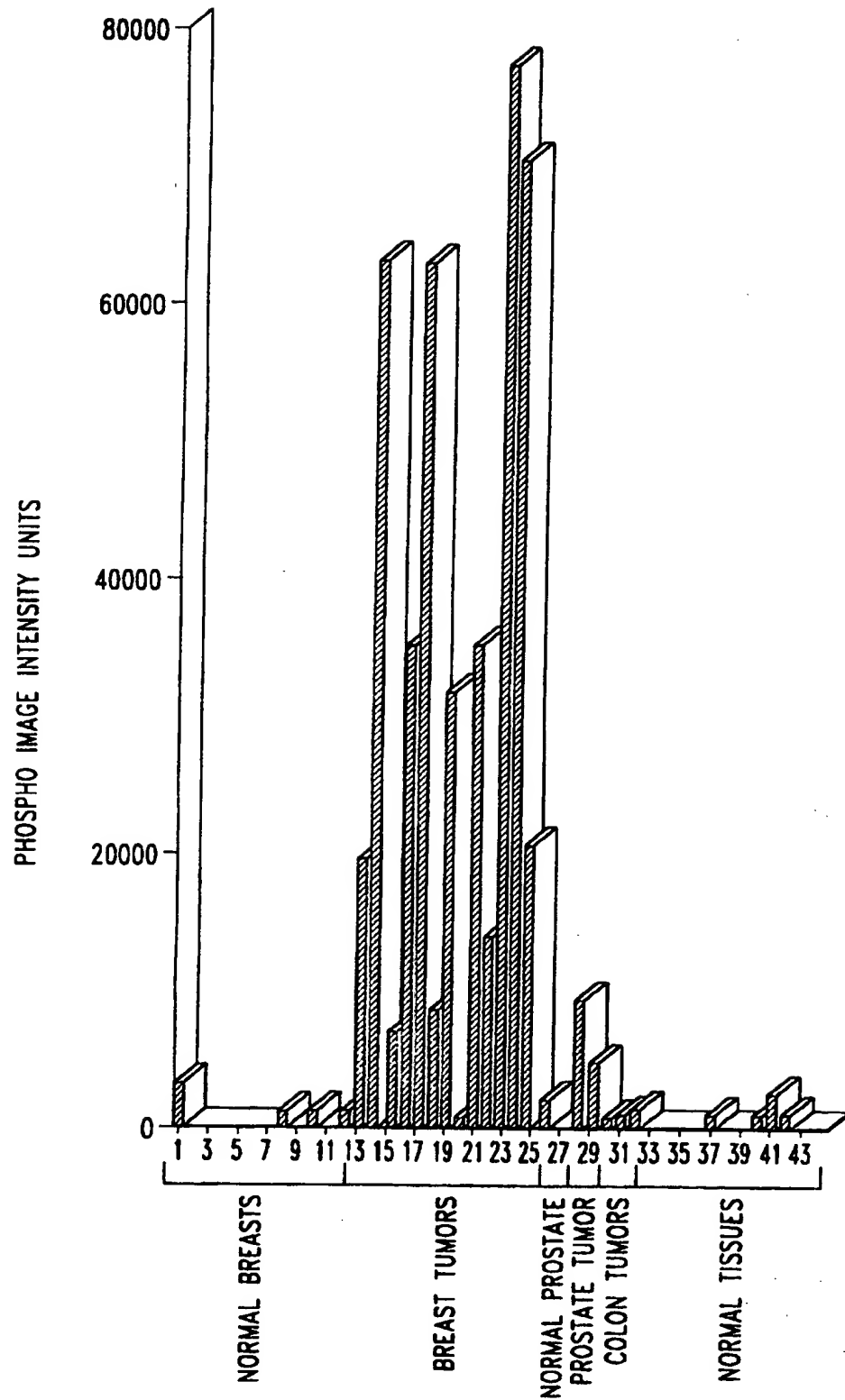


Fig. 3

4/6

GENOMIC CLONE MAP

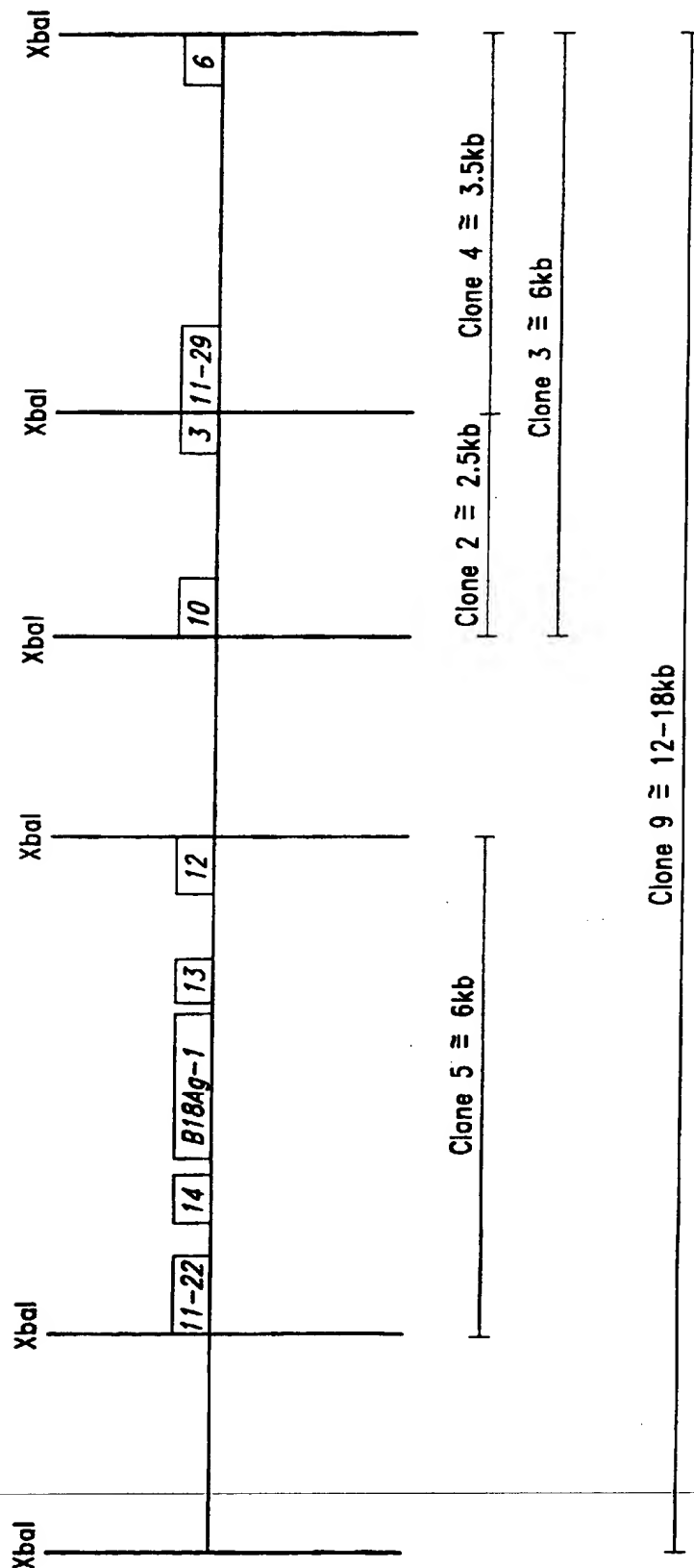
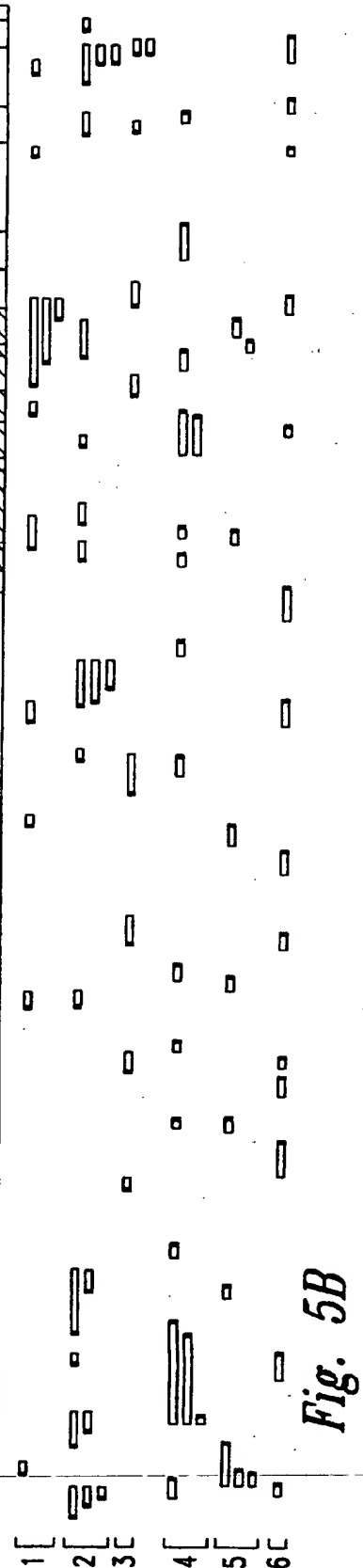
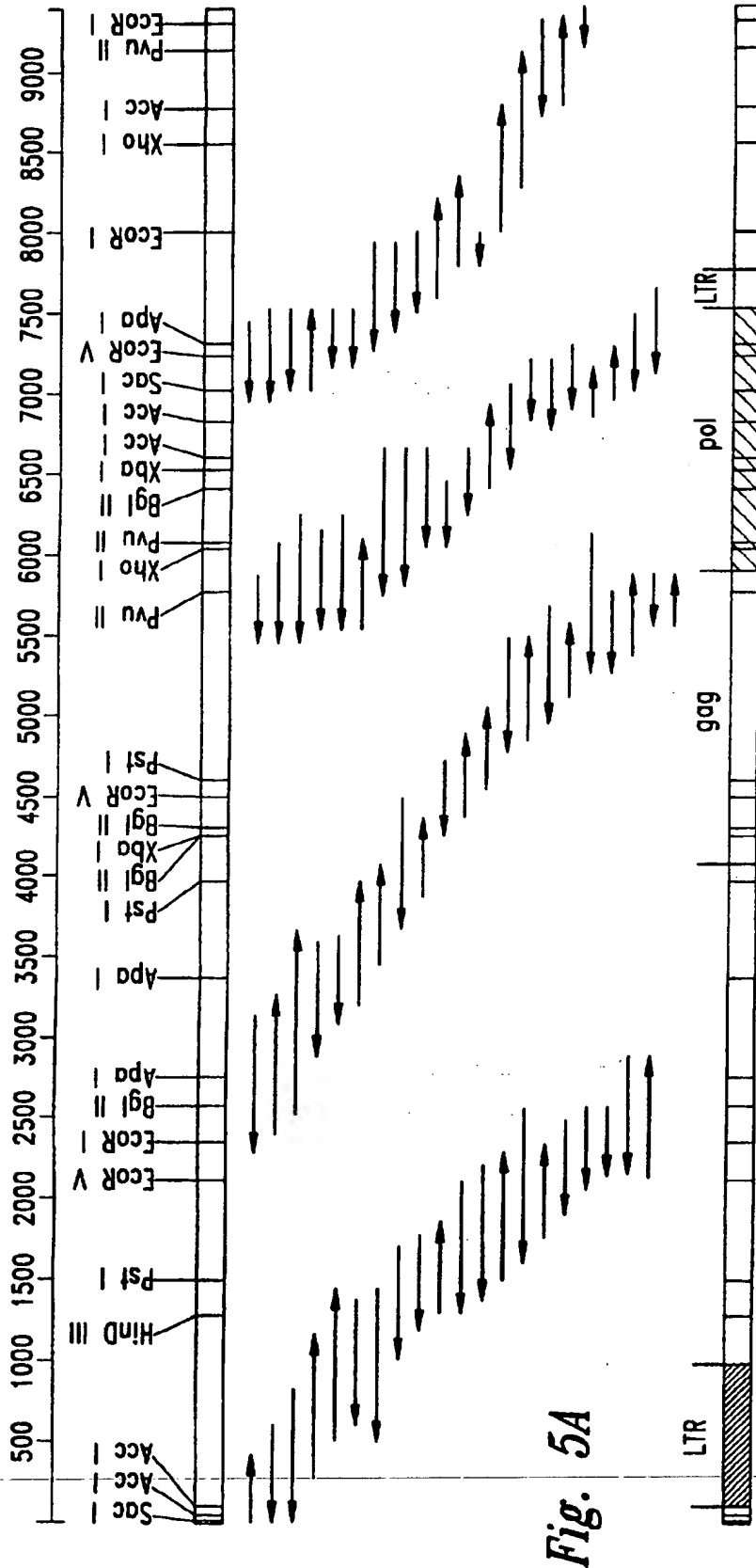


Fig. 4

5/6



6/6

NUCLEOTIDE SEQUENCE OF THE REPRESENTATIVE
BREAST-TUMOR SPECIFIC cDNA B18Ag1

TTA GAG ACC CAA TTG GGA CCT AAT TGG GAC CCA AAT TTC TCA AGT GGA	48
Leu Glu Thr Gln Leu Gly Pro Asn Trp Asp Pro Asn Phe Ser Ser Gly	
1 5 10 15	
GGG AGA ACT TTT GAC GAT TTC CAC CGG TAT CTC CTC GTG GGT ATT CAG	96
Gly Arg Thr Phe Asp Asp Phe His Arg Tyr Leu Leu Val Gly Ile Gln	
20 25 30	
GGA GCT GCC CAG AAA CCT ATA AAC TTG TCT AAG GCG ATT GAA GTC GTC	144
Gly Ala Ala Gln Lys Pro Ile Asn Leu Ser Lys Ala Ile Glu Val Val	
35 40 45	
CAG GGG CAT GAT GAG TCA CCA GGA GTG TTT TTA GAG CAC CTC CAG GAG	192
Gln Gly His Asp Glu Ser Pro Gly Val Phe Leu Glu His Leu Gln Glu	
50 55 60	
GCT TAT CGG ATT TAC ACC CCT TTT GAC CTG GCA GCC CCC GAA AAT AGC	240
Ala Tyr Arg Ile Tyr Thr Pro Phe Asp Leu Ala Ala Pro Glu Asn Ser	
65 70 75 80	
CAT GCT CTT AAT TTG GCA TTT GTG GCT CAG GCA GCC CCA GAT AGT AAA	288
His Ala Leu Asn Leu Ala Phe Val Ala Gln Ala Ala Pro Asp Ser Lys	
85 90 95	
AGG AAA CTC CAA AAA CTA GAG GGA TTT TGC TGG AAT GAA TAC CAG TCA	336
Arg Lys Leu Gln Lys Leu Glu Gly Phe Cys Trp Asn Glu Tyr Gln Ser	
100 105 110	
GCT TTT AGA GAT AGC CTA AAA GGT TTT	363
Ala Phe Arg Asp Ser Leu Lys Gly Phe	
115 120	

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/00398

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/48 C07K14/15 C07K16/10 G01N33/569 G01N33/574
G01N33/577 C12Q1/70 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 01301 A (GEN HOSPITAL CORP) 25 February 1988 see page 4, line 4 - page 22, line 11; claims; figure 1 ---	1,3,4, 11-13, 21,22, 28,29
X	JOURNAL OF VIROLOGY, vol. 69, no. 1, January 1995, pages 414-421, XP002031129 SAUTER ET AL.: "Human endogenous retrovirus K10: expression of Gag protein and detection of antibodies in patients with seminomas" see the whole document --- -/--	1,3-9, 15, 17-19, 24,26,27

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

22 May 1997

Date of mailing of the international search report

30.05.97

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Macchia, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 777 127 A (SUNI JUKKA ET AL) 11 October 1988 see the whole document ---	1,3-9, 15, 17-19, 24,26,27
X	BLOOD, vol. 86, no. 10 S1, 15 November 1995, XP000673682 WILLER ET AL.: "Transcription pattern of human endogenous retrovirus-K (HERV-K) related env sequences in patients with CML, NHL, colon cancer and mammary carcinoma" ab. 2236 see abstract ---	1,3-9, 11-13, 15, 17-19, 21,22, 24,26-29
X	WO 94 11514 A (ASTA MEDICA AG ;DIERICH MANFRED (AT); VOGETSEDER WERNER (AT)) 26 May 1994 see the whole document ---	1,3-9, 11-13, 15, 17-19, 21,22, 24,26-29
X	VIROLOGY, vol. 174, no. 1, January 1990, pages 225-238, XP000673688 WERNER ET AL.: "S71 is a phylogenetically distinct human endogenous retroviral element with structural and sequence homology to Simian Sarcoma Virus (SSV)" see page 228 - page 230; figure 2 ---	2
A		10,14
A	GENOMICS, vol. 18, 1993, pages 261-269, XP000673683 LEIB-MÖSCH: "Genomic distribution and transcription of solitary HERV-K LTRs" see page 264 - page 265; figure 3 ---	10,14
A	VIROLOGY, vol. 209, 1995, pages 550-560, XP002031131 HALTMEIER ET AL.: "Identification of S71-related human endogenous retroviral sequences with full-length pol genes" see page 556; figure 6 ---	10,14
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SCIENCE, vol. 234, 7 November 1986, pages 728-731, XP002031132 EARL ET AL.: "T-lymphocyte priming and protection against Friend Leukemia by vaccinia-retrovirus env gene recombinant" see the whole document -----</p>	24,25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/00398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8801301 A	25-02-88	EP 0318502 A JP 2500323 T	07-06-89 08-02-90
US 4777127 A	11-10-88	NONE	
WO 9411514 A	26-05-94	GB 2273099 A	08-06-94